

A Guide to Polyacrylamide Gel Electrophoresis and Detection

BIO-RAD

Part I: Theory and Product Selection5

Chapter 1 Overview5

How Protein Electrophoresis Works6

General Considerations and Workflow6

Chapter 2 Protein Electrophoresis Methods and Instrumentation9

Protein Electrophoresis Methods10

 Polyacrylamide Gel Electrophoresis (PAGE)10

 Discontinuous Native PAGE10

 SDS-PAGE11

 Other Types of PAGE12

 Blue Native PAGE (BN-PAGE)12

 Zymogram PAGE12

 Isoelectric Focusing (IEF)13

 2-D Electrophoresis13

Electrophoresis Cells and Power Supplies13

 Electrophoresis Cells13

 Power Supplies for PAGE Applications15

Chapter 3 Sample Preparation for Electrophoresis17

General Considerations19

Cell Disruption19

Protein Solubilization20

 Detergents20

 Reducing Agents20

 Chaotropic Agents21

 Buffers and Salts21

 Common Solutions for Protein Solubilization21

Removal of Interfering Substances21

Products for Sample Preparation21

Sample Quantitation (Protein Assays)22

 Protein Assays22

 SmartSpec™ Plus Spectrophotometer23

Chapter 4 Reagent Selection and Preparation25

General Considerations26

Protein Standards26

 Recombinant Standards26

 Natural Standards27

Polyacrylamide Gels27

 Polymerization27

 Percentage28

 Precast vs. Handcast29

 Format (Size and Comb Type)29

Buffer Systems and Gel Chemistries29

 Laemmli (Tris-HCl)31

 Bis-Tris31

 Tris-Acetate31

 Tris-Tricine31

 IEF31

 Zymogram31

Products for Handcasting Gels32

 Premade Buffers and Reagents32

 AnyGel™ Stands32

 Multi-Casting Chambers32

 Gradient Formers32

Chapter 5 Performing Electrophoresis35

System Setup36

Running Conditions36

 Useful Equations36

 Joule Heating36

 Other Factors Affecting Electrophoresis36

 Selecting Power Supply Settings37

 Separations Under Constant Voltage37

 Separations Under Constant Current37

 Separations Under Constant Power37

General Guidelines for Running Conditions37

Gel Disassembly and Storage37

Chapter 6 Protein Detection and Analysis39

Protein Stains40

 Total Protein Stains40

 Specific Protein Stains40

 Dodeca™ High-Throughput Stainers42

Imaging42

 Imaging Systems42

 Imaging Software44

Analysis44

 Molecular Weight (Size) Estimation44

 Quantitation45

Chapter 7 Downstream Applications47

Western Blotting (Immunoblotting)48

Gel Drying48

Electroelution48

Spot Excision (Cutting)49

Part II: Methods51

Protocols52

 Sample Preparation52

 General Tips for Sample Preparation52

 Lysis (Cell Disruption)52

 Protein Solubilization52

 Preparation for PAGE52

 Human Cells53

 Suspension Cultured Cells53

 Monolayer Cultured Cells53

 Mammalian Tissue54

 Plant Leaves54

 Microbial Cultures55

 Protein Fractions from Chromatography55

 Sample Quantitation (RC DC™ Protein Assay)56

 Standard Assay Protocol (5 ml)56

 Microcentrifuge Tube Assay Protocol (1.5 ml)56

 Handcasting Polyacrylamide Gels57

 Single-Percentage Gels57

 Pour the Resolving Gel58

 Pour the Stacking Gel58

 Gradient Gels59

 Performing Electrophoresis60

 General Protocols: SDS-PAGE60

 Total Protein Staining62

 Bio-Safe™ Coomassie Stain62

 Oriole™ Fluorescent Gel Stain62

 Flamingo™ Fluorescent Gel Stain62

 Silver Staining (Bio-Rad Silver Stain)63

 Molecular Weight Estimation63

Buffer Formulations64

 Sample Preparation Buffers64

 Gel Casting Reagents65

 Sample Buffers65

 Running Buffers66

 Buffer Components66

Part III: Troubleshooting69

Sample Preparation70

Gel Casting and Sample Loading70

Electrophoresis71

Total Protein Staining72

Evaluation of Separation73

Part IV: Appendices77

Glossary78

References and Related Reading83

Ordering Information86



PART I

Theory and Product Selection

CHAPTER 1

Overview

Protein electrophoresis is the movement of proteins within an electric field. Popular and widely used in research, it is most commonly used to separate proteins for the purposes of analysis and purification. This chapter provides a brief overview of the theory and workflow behind protein electrophoresis.

How Protein Electrophoresis Works

The term electrophoresis refers to the movement of charged molecules in response to an electric field, resulting in their separation.

In an electric field, proteins move toward the electrode of opposite charge. The rate at which they move (migration rate, in units of cm^2/Vsec) is governed by a complex relationship between the physical characteristics of both the electrophoresis system and the proteins. Factors affecting protein electrophoresis include the strength of the electric field, the temperature of the system, the pH, ion type, and concentration of the buffer as well as the size, shape, and charge of the proteins (Garfin 1990) (Figure 1.1). Proteins come in a wide range of sizes and shapes and have charges imparted to them by the dissociation constants of their constituent amino acids. As a result, proteins have characteristic migration rates that can be exploited for the purpose of separation. Protein electrophoresis can be performed in either liquid or gel-based media and can also be used to move proteins from one medium to another (for example, in blotting applications).

Over the last 50 years, electrophoresis techniques have evolved as refinements have been made to the buffer systems, instrumentation, and visualization techniques used. Protein electrophoresis can be used for a variety of applications such as purifying proteins, assessing protein purity (for example, at various stages during a chromatographic separation), gathering data on the regulation of protein expression, or determining protein size, isoelectric point (pI), and enzymatic activity. In fact, a significant number of techniques including gel electrophoresis, isoelectric focusing (IEF), electrophoretic transfer (blotting), and two-dimensional (2-D) electrophoresis can be grouped under the term “protein electrophoresis” (Rabilloud 2010). Though some information is provided about these methods in the following chapters, this guide focuses on the one-dimensional separation of proteins in polyacrylamide gels, or polyacrylamide gel electrophoresis (PAGE).

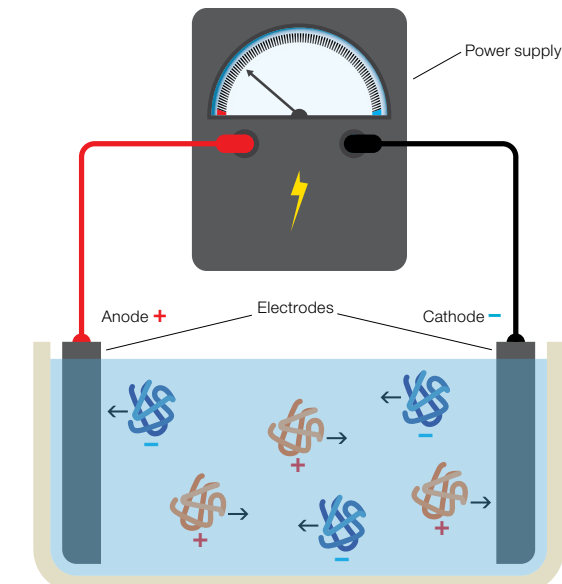


Fig. 1.1. Movement of proteins during electrophoresis.

General Considerations and Workflow

The electrophoresis workflow (Figure 1.2) involves the selection of the appropriate method, instrumentation, and reagents for the intended experimental goal. Once proteins are separated, they are available for a number of downstream applications, including enzymatic assays, further purification, transfer to a membrane for immunological detection (immunoblotting or western blotting), and elution and digestion for mass spectrometric analysis.

Protein Electrophoresis Workflow

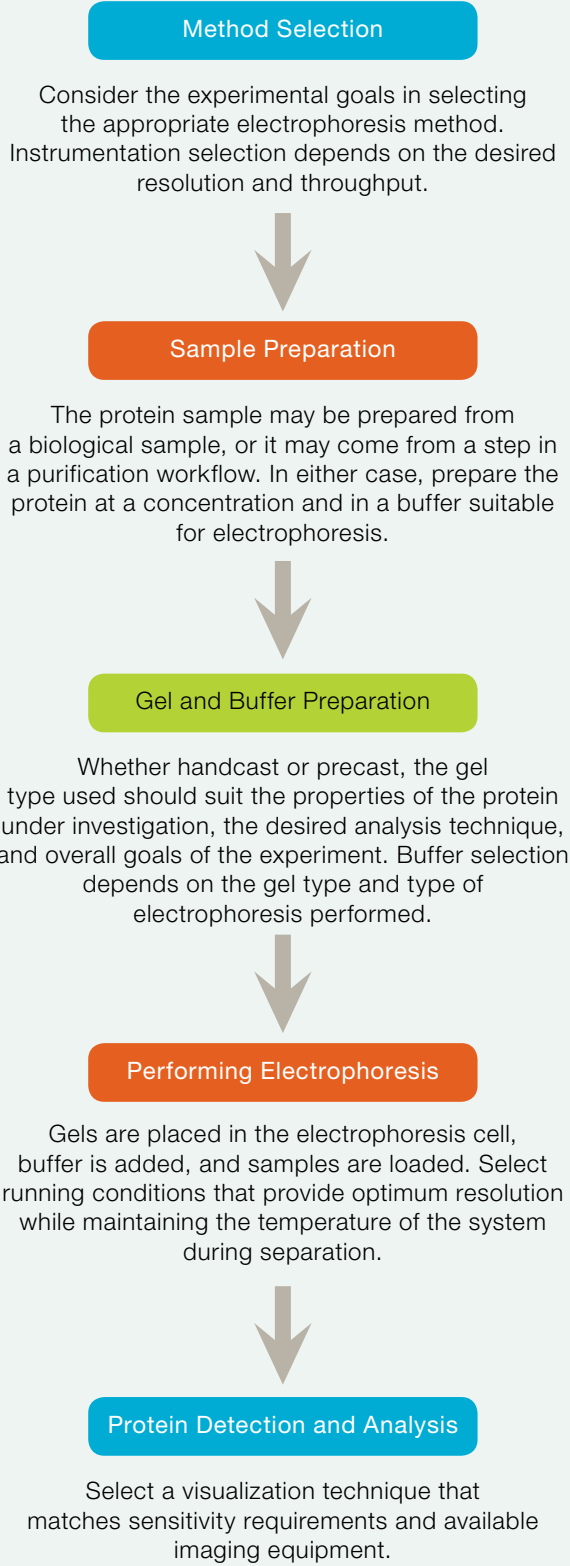


Fig. 1.2. Protein electrophoresis workflow.

Related Literature

- Protein Blotting Guide, A Guide to Transfer and Detection, bulletin 2895
- 2-D Electrophoresis for Proteomics: A Methods and Product Manual, bulletin 2651



CHAPTER 2

Protein Electrophoresis Methods and Instrumentation

Consider the experimental goals in selecting the appropriate electrophoresis method; selection of instrumentation depends on the number and volume of samples, desired resolution, and throughput. This chapter describes the most common techniques and systems in use today.

Protein Electrophoresis Methods

By choosing suitable separation matrices and corresponding buffer systems, a range of experimental objectives can be met using protein electrophoresis (Zewart and Harrington 1993).

Polyacrylamide Gel Electrophoresis (PAGE)

When electrophoresis is performed in acrylamide or agarose gels, the gel serves as a size-selective sieve during separation. As proteins move through a gel in response to an electric field, the gel's pore structure allows smaller proteins to travel more rapidly than larger proteins (Figure 2.1). For protein separation, virtually all methods use polyacrylamide as an anticonvective, sieving matrix covering a protein size range of 5–250 kD. Some less common applications such as immunoelectrophoresis and the separation of large proteins or protein complexes >300 kD rely on the larger pore sizes of agarose gels.

In most PAGE applications, the gel is mounted between two buffer chambers, and the only electrical path between the two buffers is through the gel. Usually, the gel has a vertical orientation, and the gel is cast with a comb that generates wells in which the samples are applied (Figure 2.1). Applying an electrical field across the buffer chambers forces the migration of protein into and through the gel (Hames 1998).

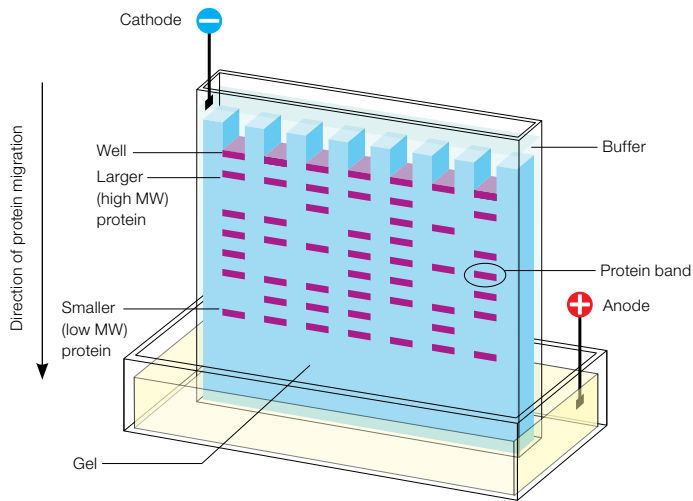


Fig. 2.1. Schematic of electrophoretic protein separation in a polyacrylamide gel. MW, molecular weight.

Two types of buffer systems can be used:

- Continuous buffer systems use the same buffer (at constant pH) in the gel, sample, and electrode reservoirs (McLellan 1982). Continuous systems are not common in protein separations; they are used mostly for nucleic acid analysis
- Discontinuous buffer systems use a gel separated into two sections (a large-pore stacking gel on top of a small-pore resolving gel, Figure 2.2) and different buffers in the gels and electrode solutions (Wheeler et al. 2004)

In gel electrophoresis, proteins do not all enter the gel matrix at the same time. Samples are loaded into wells, and the proteins that are closer to the gel enter the gel first. In continuous systems, the uniform separation matrix yields protein bands that are diffuse and poorly resolved. In discontinuous systems, on the other hand, proteins first migrate quickly through the large-pore stacking gel and then are slowed as they enter the small-pore resolving gel. As they slow down, they stack on top of one another to form a tight band, which improves resolution. Discontinuous systems also use ions in the electrophoresis buffer that sandwich the proteins as they migrate through the gel, and this tightens the protein bands even more (Figure 2.2). Discontinuous buffer systems provide higher resolution than continuous systems, and varying the buffers used in the sample, gel, and electrode chambers creates a variety of discontinuous buffer systems that can be used for a variety of applications.

Discontinuous Native PAGE

The original discontinuous gel system was developed by Ornstein and Davis (Ornstein 1964, Davis 1964) for the separation of serum proteins in a manner that preserved native protein conformation, subunit interactions, and biological activity (Vavricka 2009). In such systems, proteins are prepared in nonreducing, nondenaturing sample buffer, and electrophoresis is also performed in the absence of denaturing and reducing agents.

Data from native PAGE are difficult to interpret. Since the native charge-to-mass ratio of proteins is preserved, protein mobility is determined by a complex combination of factors. Since protein-protein interactions are retained during separation, some proteins may also separate as multisubunit complexes and move in unpredictable ways. Moreover, because native charge is preserved, proteins can migrate towards either electrode, depending on their charge. The result is that native PAGE yields unpredictable separation patterns that are not suitable for molecular weight determination.

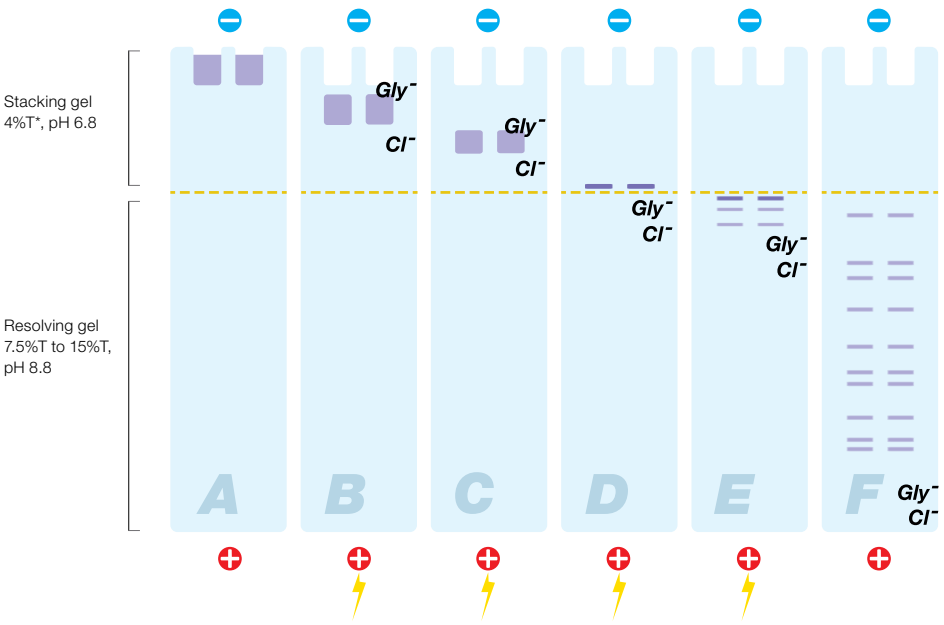


Fig. 2.2. Migration of proteins and buffer ions in a denaturing discontinuous PAGE system. A, Denatured sample proteins are loaded into the wells; B, Voltage is applied and the samples move into the gel. The chloride ions already present in the gel (leading ions) run faster than the SDS-bound proteins and form an ion front. The glycinate ions (trailing ions) flow in from the running buffer and form a front behind the proteins; C, A voltage gradient is created between the chloride and glycinate ions, which sandwich the proteins in between them; D, The proteins are stacked between the chloride and glycinate ion fronts. At the interface between the stacking and resolving gels, the percentage of acrylamide increases and the pore size decreases. Movement of the proteins into the resolving gel is met with increased resistance; E, The smaller pore size resolving gel begins to separate the proteins based on molecular weight only, since the charge-to-mass ratio is equal in all the proteins of the sample; F, The individual proteins are separated into band patterns ordered according to their molecular weights.

* %T refers to the total monomer concentration of the gel (see Chapter 4 for more information).

Nevertheless, native PAGE does allow separation of proteins in their active state and can resolve proteins of the same molecular weight.

SDS-PAGE

To overcome the limitations of native PAGE systems, Laemmli (1970) incorporated the detergent sodium dodecyl sulfate (SDS) into a discontinuous denaturing buffer system, creating what has become the most popular form of protein electrophoresis, SDS-PAGE.

When proteins are separated in the presence of SDS and denaturing agents, they become fully denatured and dissociate from each other. In addition, SDS binds noncovalently to proteins in a manner that imparts:

- An overall negative charge on the proteins. Since SDS is negatively charged, it masks the intrinsic charge of the protein it binds
- A similar charge-to-mass ratio for all proteins in a mixture, since SDS binds at a consistent rate of 1.4 g of SDS per 1 g protein (a stoichiometry of about one SDS molecule per two amino acids)
- A long, rod-like shape on the proteins instead of a complex tertiary conformation (Figure 2.3)

As a result, the rate at which SDS-bound protein migrates in a gel depends primarily on its size, enabling molecular weight estimation.

The original Laemmli system incorporated SDS in the gels and buffers, but SDS is not required in the gel. SDS in the sample buffer is sufficient to saturate proteins, and the SDS in the cathode buffer maintains the SDS saturation during electrophoresis. Precast gels (manufactured gels such as Bio-Rad's Mini-PROTEAN® and Criterion™ gels) do not include SDS and so can be used for either native or SDS-PAGE applications. A range of gel and buffer combinations can be used for native and SDS-PAGE, each with its own advantages (see Chapter 4 for more details).

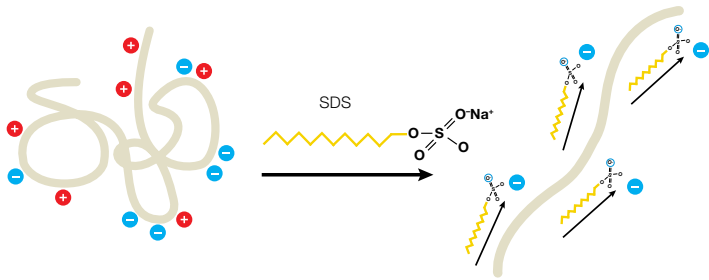


Fig. 2.3. Effect of SDS on the conformation and charge of a protein.

Related Literature

Gel Electrophoresis: Separation of Native Basic Proteins by Cathodic, Discontinuous Polyacrylamide Gel Electrophoresis, bulletin 2376

Related Literature

2-D Electrophoresis for Proteomics: A Methods and Product Manual, bulletin 2651

TABLE OF CONTENTS

Links

Experion Automated Electrophoresis System

Experion Protein Analysis Kits

Coomassie Stains

Coomassie Brilliant Blue G-250 Stain

Coomassie Brilliant Blue R-250 Stain

Automated Electrophoresis

Automated electrophoresis systems use microfluidic technology to integrate electrophoretic separation, fluorescence detection, and data analysis within a single platform. Using much smaller sample and reagent quantities and far less time than standard analysis methods, automated electrophoresis systems like Bio-Rad's Experion™ system can be used in applications such as purity and yield assessment in protein purification and protocol optimization workflows. However, samples cannot be recovered from automated separations, and downstream applications are thus not possible.

The Experion system employs LabChip microfluidic technology to automate complete protein and nucleic acid electrophoresis and analyze up to 12 samples in a single 30–40 min automated step (analysis of a single sample is accomplished in as little as 90 sec).

Protein analysis with the Experion system is achieved with the Experion Pro260 analysis kit, which supplies the microfluidic chips, reagents, and instructions required to separate and analyze 10–260 kD proteins under denaturing conditions. The sensitivity of the Experion Pro260 analysis kit is comparable to that of colloidal Coomassie (Brilliant) Blue staining of SDS-PAGE gels.

Experion software converts a fluorescent signal into an electropherogram (a plot of fluorescence vs. time) and a densitometric gel-like image, or virtual gel. Each lane in the virtual gel corresponds to a different sample, and the peaks in the electropherogram correspond to bands in the virtual gel. The results of data analysis are also presented in a table at the end of analysis.

The types of data available include the following:

- Protein size (or mass, in kD)
- Protein concentration (in ng/μl): relative and absolute concentration
- Consider protein purity (% of the total sample) as an alternative

The Experion system provides single-step quantitation and size determination (sizing) of protein samples useful for quality control, protein purity and stability analysis, protocol optimization, and evaluation of recombinant protein expression.



The Experion system. The system includes: **1**, automated electrophoresis station; **2**, priming station; **3**, vortex station used only for nucleic acid analysis; **4**, system operation and data analysis tools (software); and **5**, analysis kits, which include the chips (a) and reagents (b) for protein (Pro260 kit), standard-sensitivity RNA (StdSens kit), high-sensitivity RNA (HighSens kit), and DNA (DNA 1K and 12K kits) analyses.

Other Types of PAGE

Blue Native PAGE (BN-PAGE)

BN-PAGE is used to separate and characterize large protein complexes in their native and active forms. Originally described by Schagger and von Jagow (1987), this technique relies on the solubilization of protein complexes with mild, neutral detergents and the binding of negatively charged Coomassie (Brilliant) Blue G-250 stain to their surfaces. This imparts a high charge-to-mass ratio that allows the protein complexes to migrate to the anode as they do in SDS-PAGE. Coomassie Blue does not, however, denature and dissociate protein complexes the way SDS does. High-resolution separation is achieved by electrophoresis into an acrylamide gradient with decreasing pore

sizes; the protein complexes become focused at the corresponding pore size limit (Nijtmans et al. 2002, Reisinger and Eichacker 2008).

Zymogram PAGE

Zymogram PAGE is used to detect and characterize collagenases and other proteases within the gel. Gels are cast with gelatin or casein, which acts as a substrate for the enzymes that are separated in the gel under nonreducing conditions. The proteins are run with denaturing SDS in order to separate them by molecular weight. After renaturing the enzymes and then allowing them to break down the substrate, zymogram gels are stained with Coomassie (Brilliant) Blue R-250 stain, which stains the substrate while leaving clear areas around active proteases.

Isoelectric Focusing (IEF)

IEF combines the use of an electric field with a pH gradient to separate proteins according to their pI. It offers the highest resolution of all electrophoresis techniques (Westermeyer 2004).

When a protein moves through a pH gradient, its net charge changes in response to the pH it encounters. Under the influence of an electric field, a protein in a pH gradient migrates to a pH where its net charge is zero (the protein's pI). If the protein moves out of that position, it acquires a charge and is forced back to the zero-charge position (Figure 2.4). This focusing is responsible for the high resolution of IEF. pI values of proteins usually fall in the range of pH 3–11.

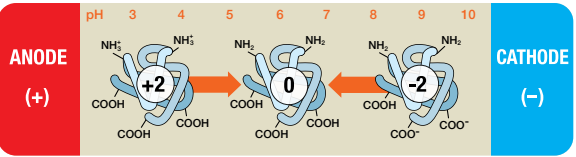


Fig. 2.4. Isoelectric focusing. A protein is depicted in a pH gradient in an electric field. A pH gradient formed by ampholyte molecules under the influence of an electric field is indicated. The gradient increases from acidic (pH 3) at the anode to basic (pH 10) at the cathode. The hypothetical protein in the drawing bears a net charge of +2, 0, or –2, at the three positions in the pH gradient shown. The electric field drives the protein toward the cathode when it is positively charged and toward the anode when it is negatively charged, as shown by the arrows. At the pI, the net charge on the protein is zero, so it does not move in the field. The protein loses protons as it moves toward the cathode and becomes progressively less positively charged. Conversely, the protein gains protons as it moves toward the anode and becomes less negatively charged. When the protein becomes uncharged (pI), it ceases to move in the field and becomes focused.

Two methods are used to generate a stable, continuous pH gradient between the anode and cathode:

- Carrier ampholytes — heterogeneous mixtures of small (300–1,000 Da) conductive polyamino-polycarboxylate compounds that carry multiple charges with closely spaced pI values. When voltage is applied across an ampholyte-containing solution or gel, the ampholytes align themselves according to their pIs and buffer the pH in their proximity, establishing a pH gradient. Ampholytes can be used in gels (for example, tube gels or vertical gels) or in solution (for example, liquid-phase IEF)
- Immobilized pH gradients (IPG) strips — formed by covalently grafting buffering groups to a polyacrylamide gel backbone. A gradient of different buffering groups generates a stable pH gradient that can be tailored for different pH ranges and gradients (Bjellquist et al. 1982)

Bio-Rad's PROTEAN® i12™ IEF system provides individual lane control for up to 12 IPG strips, making it possible to run different sample types, different pH gradients, and multiple protocols at the same time. IEF can be run under either native or denaturing conditions. Native IEF retains protein structure and enzymatic activity. However, denaturing IEF is performed in the presence of high concentrations of urea, which dissociates proteins into individual subunits and abolishes secondary and tertiary structures. Whereas native IEF may be a more convenient option because it can be performed with a variety of precast gels, denaturing IEF often offers higher resolution and is more suitable for the analysis of complex protein mixtures.

2-D Electrophoresis

The sequential application of different electrophoresis techniques produces a multi-dimensional separation. The most common 2-D technique (O'Farrell 1975) subjects protein samples first to denaturing IEF on a tube gel or IPG gel strip (for separation by pI), then to SDS-PAGE for further separation by molecular weight. High-resolution 2-D methods enable separation of thousands of polypeptides in a single slab gel. The resulting spots can be visualized by gel staining, or they can be transferred to a membrane support for total protein staining or analysis with specific antibody detection. For more details, refer to 2-D Electrophoresis for Proteomics (bulletin 2651).

Electrophoresis Cells and Power Supplies

Vertical electrophoresis cells are plastic boxes with anode and cathode buffer compartments that contain electrodes (Figure 2.5). The electrodes (typically platinum wire) connect to a jack attached to a power supply. The gels are held vertically between the electrode chambers during electrophoresis (Andrews 1986).

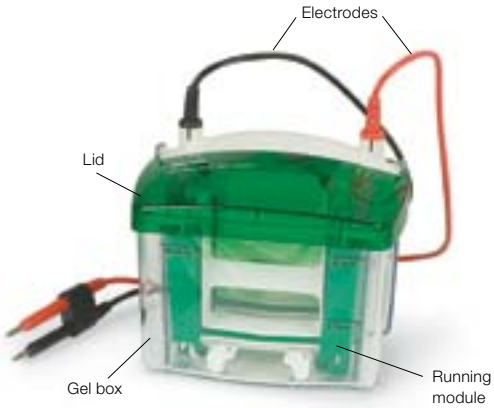


Fig. 2.5. Components of a vertical electrophoresis cell.

Links

Mini Format 1-D Electrophoresis Systems

Mini-PROTEAN Precast Gels

Mini-PROTEAN Tetra Cell

Mini-PROTEAN 3 Dodeca Cell

Midi Format 1-D Electrophoresis Systems

Criterion Precast Gels

Criterion Cell

Criterion Dodeca Cell

Large-Format 1-D Electrophoresis Systems

PROTEAN II xi Cell

PROTEAN II XL Cell

PROTEAN II xi and XL Multi-Cells

PROTEAN Plus Dodeca Cell

PROTEAN i12 IEF System

Related Literature

- Mini-PROTEAN Tetra Cell Brochure, bulletin 5535
- Criterion Precast Gel System Brochure, bulletin 2710
- PROTEAN II xi/XL Cells Product Information Sheet, bulletin 1760

Vertical electrophoresis cells are made in different size formats to accommodate different gels sizes. Deciding which cell to use depends on the requirements for speed, resolution, and throughput (both the number of samples and gels) as well as the volume of sample available (Table 2.1).

- Mini-format systems — accommodate small gels (up to 8.6 x 6.7 cm). The short separation distance maximizes the electrical field strength (V/cm) to yield rapid separations with moderate resolution. Use these systems for rapid analysis, method development, or when sample volumes are limited. The Mini-PROTEAN® system includes the Mini-PROTEAN Tetra cell (with a

capacity of up to four gels) and the high-throughput Mini-PROTEAN® 3 Dodeca™ cell (for running up to 12 gels); both cells are compatible with Mini-PROTEAN precast gels

- Midi-format systems — accommodate 13.3 x 8.7 cm gels and offer rapid runs with more samples per gel and enhanced separation over mini-format gels. The Criterion™ system includes the Criterion cell (for 1–2 gels) and the high-throughput Criterion™ Dodeca™ cell (for 1–12 gels); both cells are compatible with Criterion precast gels
- Large-format systems — accommodate large gels (up to 20 x 18.3 cm for the PROTEAN® II system

and 20 x 20.5 cm for the PROTEAN Plus System) and offer maximum resolution. The PROTEAN II system provides a choice of glass plates, spacer, and sandwich clamps to cast two gel lengths: 16 or 20 cm. The PROTEAN® Plus Dodeca™ cell allows maximum throughput with the capability to run up to 12 gels at a time

Power Supplies for PAGE Applications

Power supplies are available to meet the power requirements of numerous applications. The choice

of power supply for PAGE applications usually depends on the size and number of gels being run. Table 2.2 compares the Bio-Rad PowerPac power supplies recommended for vertical electrophoresis applications.

- Use the PowerPac Basic or PowerPac HC high-current power supply for mini-format vertical PAGE applications
- Use the PowerPac HV high-voltage or PowerPac Universal power supply for large-format vertical PAGE applications
- Use the PowerPac HC power supply for applications that require high currents, such as PAGE with the high-throughput Dodeca cells

Related Literature

- PowerPac Basic 300 V Power Supply Flier, bulletin 2881
- PowerPac HC High-Current Power Supply Flier, bulletin 2882
- PowerPac Universal Power Supply Brochure, bulletin 2885
- PowerPac HV Power Supply Brochure, bulletin 3189

Table 2.1. Vertical electrophoresis system selection guide.





| | Mini-PROTEAN System | Criterion System | PROTEAN II System | PROTEAN Plus System |
|--|---|--|--|---|
| |  |  |  |  |
| Advantages | Run 1–4 precast or handcast gels in the Mini-PROTEAN Tetra cell and up to 12 gels in the Mini-PROTEAN Dodeca cell in mini format Wing clamp assembly allows faster setup and leak-free operation | Fast setup with drop-in gel and cell design (precast or handcast) Run 1–2 precast Criterion or handcast gels in the Criterion cell and up to 12 gels in the Criterion Dodeca cell Integrated upper buffer chamber allows leak-free operation | Large-format gel system offers greater resolution over smaller formats Can accommodate up to 4 gels and is available in xi or XL formats for running a variety of gel sizes Multi-cell is available for running up to 6 gels | Offers maximum resolution in a single gel and the longest range of separation (with the ability to run up to 12 gels) Specifically for the second dimension of 2-D electrophoresis |
| Compatible Gel Formats | | | | |
| Precast | Mini-PROTEAN precast gels Ready Gel® precast gels | Criterion precast gels | | |
| Handcast | Ready Gel empty cassettes Mini-PROTEAN casting plates | Criterion empty cassettes | PROTEAN II casting plates | PROTEAN Plus casting equipment |
| Electrophoresis Cells | Mini-PROTEAN Tetra Mini-PROTEAN 3 Dodeca | Criterion Criterion Dodeca | PROTEAN II xi/XL PROTEAN II xi/XL multi-cells | PROTEAN Plus Dodeca |
| Precast Gel Dimensions | | | | |
| W x L x thickness | Mini-PROTEAN precast gels: 8.6 x 6.7 x 0.1 cm Ready Gel precast gels: 8.3 x 6.4 x 0.1 cm | Criterion precast gels: 13.3 x 8.7 x 0.1 cm | | |
| Cassette Dimensions (for Handcasting Gels) | 10.0 x 8.0 cm | 15.0 x 10.6 cm | 20.0 x 18.3 cm | 18.5 x 20.5 cm 20.0 x 20.5 cm 20.0 x 20.5 cm |
| Compatible Transfer Systems | | | | |
| Wet/tank transfer | Mini Trans-Blot® cell Criterion blotter Trans-Blot® cell | Criterion wire blotter Criterion plate blotter Trans-Blot cell Trans-Blot Plus cell | Trans-Blot cell Trans-Blot Plus cell | Trans-Blot Plus cell |
| Semi-dry transfer | Trans-Blot® Turbo™ system Trans-Blot SD cell | Trans-Blot Turbo system Trans-Blot SD cell | | |

Table 2.2. PowerPac™ power supplies selection guide.

| Technique and Recommended Apparatus | PowerPac Power Supply |
|---|--|
| Laemmli (SDS), O'Farrell Second Dimension (SDS) Mini-PROTEAN Tetra cell Criterion cell PROTEAN II xi cell PROTEAN II XL cell | Basic or HC Basic or HC HV or Universal HV or Universal |
| High-Throughput Electrophoresis Mini-PROTEAN 3 Dodeca cell Criterion Dodeca cell PROTEAN II xi/XL multi-cell PROTEAN Plus Dodeca cell | HC or Universal HC or Universal Universal HC or Universal |
| Western Blotting Mini Trans-Blot cell Criterion blotter Wire electrodes Plate electrodes Trans-Blot cell Wire electrodes Plate electrodes High-intensity transfer Trans-Blot Plus cell Trans-Blot SD cell Protein DNA/RNA | HC HC HC HC HC HC HC HC HC HC |



Fig. 2.6. PowerPac power supplies.

Preparative Electrophoresis

Preparative electrophoresis techniques separate large amounts of protein (nanogram to gram quantities) for the purposes of purification or fractionation (to reduce sample complexity). The same principles that are applied for analytical work can be applied for preparative work.

PAGE

Preparative PAGE can be accomplished using a standard slab gel or special instrumentation. With the slab gel, a single preparative or “prep” well is cast, which allows a large volume of a single sample to be applied within one well. With this approach, the separated protein is retained within the gel for further analysis or purification (for example, by electroelution).

Alternatively, continuous-elution gel electrophoresis using the Model 491 prep cell or mini prep cell yields high-resolution separations and proteins in liquid fractions, ready for downstream use.

Liquid-Phase IEF

Liquid-phase IEF devices, such as the Rotofor® cell, mini Rotofor cell, and MicroRotofor™ cell, fractionate proteins in free solution according to their pI. This nondenaturing technique produces fractions that can be collected, pooled, and even refractionated for further purification. Fractionation by liquid-phase IEF is particularly useful for proteins that do not separate well in gel-based IEF media. The pH gradient used for separation is generated by ampholytes, allowing a continuous and customizable pH gradient to be formed.

Combination Approaches (2-D Separations)

Preparative IEF and PAGE can be combined (for separation on multiple dimensions) for even greater separation.

Links

- Preparative Electrophoresis
- Power Supplies
- PowerPac Universal Power Supply
- PowerPac HC High-Current Power Supply
- PowerPac HV High-Voltage Power Supply
- PowerPac Basic Power Supply
- Model 491 Prep Cell and Mini Prep Cell
- Rotofor and Mini Rotofor Cells
- MicroRotofor Cell



CHAPTER 3

Sample Preparation for Electrophoresis

Sample preparation involves the extraction and solubilization of a protein sample that is free of contaminants and that has a total protein concentration suitable for electrophoresis. The quality of sample preparation can greatly affect the quality of the data that are generated. General guidelines and some of the most common methods for protein sample preparation are provided in this chapter.

Sample Preparation Workflow

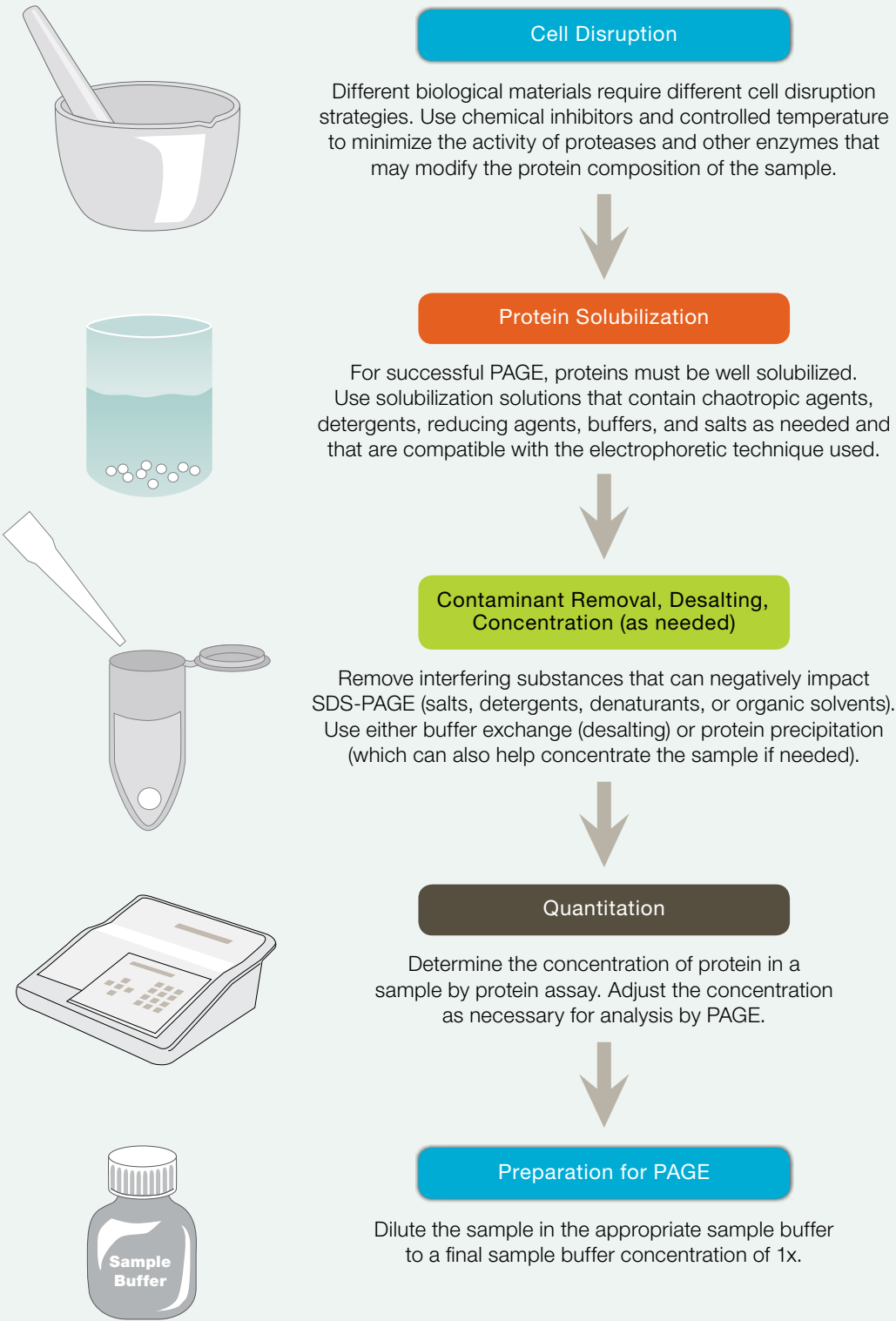


Fig. 3.1. Protein sample preparation workflow.

General Considerations

Due to the great diversity of protein sample types and sources, no single sample preparation method works with all proteins; for any sample, the optimum procedure must be determined empirically. However, the following general sample preparation guidelines should be kept in mind to avoid a number of common pitfalls during sample preparation for protein electrophoresis (Posch et al. 2006):

- Keep the sample preparation workflow as simple as possible (increasing the number of sample handling steps may increase variability)
- With cell or tissue lysates, include protease inhibitors to minimize artifacts generated by proteolysis; protease inhibitors are generally not required for samples like serum or plasma
- Determine the amount of total protein in each sample using a protein assay that is compatible with chemicals in your samples
- Solubilize proteins in a buffer that is compatible with the corresponding electrophoresis technique
- Use protein extracts immediately or aliquot them into appropriately sized batches and store them at -80°C to avoid freeze-thaw cycles

Cell Disruption

The effectiveness of a cell disruption method determines the accessibility of intracellular proteins for extraction and solubilization (Huber et al. 2003). Different biological materials require different cell disruption strategies, which can be divided into two main categories: gentle and harsher methods (Table 3.1).

- Use gentle cell disruption protocols when the sample consists of cells that lyse easily, such as red blood cells or tissue culture cells
- Use harsher methods, which are based mainly on mechanical rupture (Goldberg 2008), with biological materials that have tough cell walls (for example, plant cells, tissues, and some microbes)
- When working with a new sample, use at least two different cell disruption protocols and compare their efficiency in terms of yield (by protein assay) and qualitative protein content (by SDS-PAGE)
- Optimize the power settings of mechanical rupture systems and incubation times for all lysis approaches
- Mechanical cell lysis usually generates heat; use cooling where required to avoid overheating the sample

Table 3.1. Suitability of cell disruption methods to various sample types.

| | | | Yeast, Algae, Fungi | | Green Plant Material | Soft Tissues | Mammalian Cell Culture |
|---------------------------|--|----------|---------------------------|-------|----------------------------|-----------------|------------------------------|
| Technique | Description | Bacteria | | Seeds | | | |
| Gentle Methods | | | | | | | |
| Osmotic lysis | Suspension of cells in hypotonic solution; cells swell and burst, releasing cellular contents | — | — | — | — | — | • |
| Freeze-thaw lysis | Freezing in liquid nitrogen and subsequent thawing of cells | — | — | — | — | — | • |
| Detergent lysis | Suspension of cells in detergent-containing solution to solubilize the cell membrane; this method is usually followed by another disruption method, such as sonication | — | — | — | — | — | • |
| Enzymatic lysis | Suspension of cells in iso-osmotic solutions containing enzymes that digest the cell wall (for example, cellulase and pectinase for plant cells, lyticase for yeast cells, and lysozyme for bacterial cells); this method is usually followed by another disruption method, such as sonication | • | • | — | • | — | — |
| Harsher Methods | | | | | | | |
| Sonication | Disruption of a cell suspension, cooled on ice to avoid heating and subjected to short bursts of ultrasonic waves | • | • | — | — | — | • |
| French press | Application of shear forces by forcing a cell suspension through a small orifice at high pressure | • | • | — | — | — | • |
| Grinding | Breaking cells of solid tissues and microorganisms with a mortar and pestle; usually, the mortar is filled with liquid nitrogen and the tissue or cells are ground to a fine powder | • | • | • | • | • | — |
| Mechanical homogenization | Homogenization with either a handheld device (for example, Dounce and Potter-Elvehjem homogenizers), blenders, or other motorized devices; this approach is best suited for soft, solid tissues | — | — | — | • | • | — |
| Glass-bead homogenization | Application of gentle abrasion by vortexing cells with glass beads | • | • | — | — | — | • |

All cell disruption methods cause the release of compartmentalized hydrolases (phosphatases, glycosidases, and proteases) that can alter the protein composition of the lysates. In experiments where relative amounts of protein are to be analyzed, or in experiments involving downstream immunodetection, the data are only meaningful when the protein composition is preserved. Avoid enzymatic degradation by using one or a combination of the following techniques:

- Disrupt the sample or place freshly disrupted samples in solutions containing strong denaturing agents such as 7–9 M urea, 2 M thiourea, or 2% SDS. In this environment, enzymatic activity is often negligible
- Perform cell disruption at low temperatures to diminish enzymatic activity
- Lyse samples at pH >9 using either sodium carbonate or Tris as a buffering agent in the lysis solution (proteases are often least active at basic pH)
- Add a chemical protease inhibitor to the lysis buffer. Examples include phenylmethylsulfonyl fluoride (PMSF), aminoethyl-benzene sulfonyl fluoride (AEBSF), tosyl lysine chloromethyl ketone (TLCK), tosyl phenyl chloromethyl etone (TPCK), ethylenediaminetetraacetic acid (EDTA), benzamidine, and peptide protease inhibitors (for example, leupeptin, pepstatin, aprotinin, and bestatin). For best results, use a combination of inhibitors in a protease inhibitor cocktail
- If protein phosphorylation is to be studied, include phosphatase inhibitors such as fluoride and vanadate

Following cell disruption:

- Check the efficacy of cell wall disruption by light microscopy
- Centrifuge all extracts extensively (20,000 x g for 15 min at 15°C) to remove any insoluble material; solid particles may block the pores of the gel

Protein Solubilization

Protein solubilization is the process of breaking interactions involved in protein aggregation, for example, disulfide bonds, hydrogen bonds, van der Waals forces, ionic interactions, and hydrophobic interactions (Rabilloud 1996). If these interactions are not prevented, proteins can aggregate or precipitate, resulting in artifacts or sample loss. For successful PAGE, proteins must be well solubilized.

Ideally, cell lysis and protein solubilization are carried out in the sample buffer that is recommended for the particular electrophoresis technique, especially when native electrophoresis is the method of choice.

If this is not possible or desirable, proteins must be prepared in sample solubilization solutions that typically contain a number of compounds, including chaotropic agents, detergents, reducing agents, buffers, salts, and ampholytes. These are chosen from a small list of compounds that meet the requirements, both electrically and chemically, for compatibility with the electrophoretic technique being used. In these cases, the sample will have to be diluted with concentrated electrophoresis sample buffer to yield a 1x final buffer concentration.

Detergents

Detergents are classified as nonionic, zwitterionic, anionic, and cationic, and they disrupt hydrophobic interactions between and within proteins (Luche et al. 2003). Some proteins, especially membrane proteins, require detergents for solubilization during isolation and to maintain solubility. Nonionic detergents such as NP-40 and Triton X-100 are not very effective at solubilizing hydrophobic proteins; zwitterionic detergents such as CHAPS and sulfobetaines (for example, SB 3-10 or ASB-14) provide higher solubilization efficiency, especially for integral membrane proteins. Sample preparation for PAGE commonly uses the anionic detergent SDS, which is unparalleled in its ability to efficiently and rapidly solubilize proteins.

Reducing Agents

Thiol reducing agents such as 2-mercaptoethanol (βME) and dithiothreitol (DTT) disrupt intramolecular and intermolecular disulfide bonds and are used to achieve complete protein unfolding and to maintain proteins in their fully reduced states (Figure 3.2).

βME is volatile, evaporates from solution, and reduces protein disulfide bonds by disulfide exchange. There is an equilibrium between free thiols and disulfides, so βME is used in large excess in sample buffers to

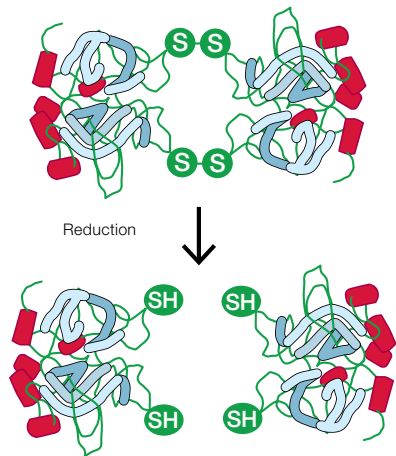


Fig. 3.2. Reduction of proteins with DTT.

drive the equilibrium reaction toward completion. If the concentration of βME drops and proteins reoxidize, fuzzy or spurious artifactual bands may result.

DTT is less volatile and is altered during the disulfide reduction reaction to form a ring structure from its original straight chain. The equilibrium favors protein reduction, so lower concentrations of DTT are needed (higher concentrations are recommended for proteins with large numbers of disulfide bonds).

Phosphines such as tributylphosphine (TBP) and Tris-carboxyethylphosphine (TCEP)* offer an alternative to thiols as reducing agents because they can be used at lower concentrations and over a wider pH range than the sulfhydryl reductants.

Chaotropic Agents

Chaotropic compounds such as urea disrupt hydrogen bonds and hydrophobic interactions both between and within proteins. When used at high concentrations, they destroy secondary protein structure and bring proteins into solution that are not otherwise soluble.

Urea and substituted ureas like thiourea improve solubilization of hydrophobic proteins. Currently, the best solution for denaturing electrophoresis is a combination of 7 M urea and 2 M thiourea in combination with appropriate detergents like CHAPS.

Samples containing urea and thiourea can be used in SDS-PAGE when diluted with SDS-PAGE sample buffer. The protein solution should not be heated above 37°C because urea and thiourea get hydrolyzed (to cyanate and thiocyanate, respectively) and modify amino acids on proteins (carbamylation), giving rise to artifactual charge heterogeneity.

Buffers and Salts

Both pH and ionic strength influence protein solubility, making buffer choice important, especially when native electrophoresis conditions are required. Many proteins are more soluble at higher pH; therefore, Tris base is often included to elevate the pH. However, proteins differ in their solubility at different pH values, so different buffers can extract different sets of proteins. The choice of buffer and pH of the sample preparation solution can strongly influence which proteins show up in a separation.

Even in the presence of detergents, some proteins have stringent salt requirements to maintain their solubility, but salt should be present only if it is an absolute requirement. Excess salt in SDS-PAGE samples causes

*TCEP is included in Bio-Rad’s XT sample buffers. Although TCEP can be added to SDS-PAGE sample buffer, it must first be neutralized with NaOH; otherwise, it will hydrolyze proteins.

fuzzy bands and narrowing of gel lanes toward the bottom of the gel. If the ionic strength is very high, no bands will appear in the lower part of the gel (a vertical streak will appear instead) and the dye front will be wavy instead of straight. Deionize any sample with a total ionic strength over 50 mM using columns such as Micro Bio-Spin™ columns, which contain 10 mM Tris at a pH suitable for SDS-PAGE.

Common Solutions for Protein Solubilization

Ideally, cell lysis and protein solubilization are carried out in the sample buffer that is recommended for the particular electrophoresis technique, especially for native electrophoresis. If this is not possible or desirable, dilute the protein solution with concentrated electrophoresis sample buffer to yield a 1x final buffer concentration.

Formulas for various sample buffers are provided in Part II of this guide.

Removal of Interfering Substances

Success or failure of any protein analysis depends on sample purity. Interfering substances that can negatively impact SDS-PAGE include salts, detergents, denaturants, or organic solvents (Evans et al. 2009). Highly viscous samples indicate high DNA and/or carbohydrate content, which may also interfere with PAGE separations. In addition, solutions at extreme pH values (for example, fractions from ion exchange chromatography) diminish the separation power of most electrophoresis techniques. Use one of the following methods as needed to remove these contaminants:

- Protein precipitation — the most versatile method to selectively separate proteins from other contaminants consists of protein precipitation by trichloroacetic acid (TCA)/acetone followed by resolubilization in electrophoresis sample buffer. A variety of commercial kits can simplify and standardize laboratory procedures for protein isolation from biological samples
- Buffer exchange — size exclusion chromatography is another effective method for removing salts, detergents, and other contaminants

Products for Sample Preparation

Bio-Rad’s solution to successful and reproducible sample preparation is the MicroRotor™ lysis kits (Figure 3.3), which provide cell lysis and protein extraction protocols tailored to the specific needs of different sample sources. All four kits are based on the same chaotropic protein solubilization buffer (PSB), which contains nondetergent sulfobetaine 201 (NDSB 201) and urea, thiourea, and CHAPS for particularly effective solubilization (Vuillard et al. 1995).

Links

- Micro Bio-Spin 6 and Micro Bio-Spin 6 Columns
- MicroRotor Lysis Kits

The kits generate total protein samples that are ready to be applied to SDS-PAGE, IEF, and 2-D gel electrophoresis. Different sample types have different requirements for effective cell disruption, and all four kits combine PSB with other elements to accommodate these specific needs.

- MicroRotor™ cell lysis kit (mammal) — for use with samples from mammalian sources, but it may also be applied to other animal tissues
- MicroRotor cell lysis kit (bacteria) — for use with both gram-negative and gram-positive bacterial cultures
- MicroRotor cell lysis kit (plant) — for use with soft plant tissues and cultured cells
- MicroRotor cell lysis kit (yeast) — for use with yeast cultures (~60 µl wet cell pellet)



Fig. 3.3. MicroRotor cell lysis kit.

For contaminant removal Bio-Rad offers the following (Figure 3.4):

- ReadyPrep™ 2-D cleanup kit — uses a modification of the traditional TCA protein precipitation protocol. The kit offers quantitative protein recovery but also ensures easy and reproducible removal of interfering substances
- Bio-Spin® and Micro Bio-Spin 6 columns — provide rapid salt removal in an easy-to-use spin-column format. Accommodating up to 100 µl of sample, these columns remove compounds <6 kD; proteins can be eluted in electrophoresis sample buffer

Sample Quantitation (Protein Assays)

Determine the concentration of protein in a sample (Berkelman 2008) by using protein assays to:

- Ensure that the amount of protein to be separated is appropriate for the lane dimensions and visualization method
- Facilitate comparison among similar samples; image-based analysis is simplified when equivalent quantities of proteins have been loaded in the lanes of the gel

The most commonly used protein assays are colorimetric assays in which the presence of protein causes a color change that can be measured with a spectrophotometer (Sapan et al. 1999, Noble and Bailey 2009). All protein assays utilize a dilution series of a known protein (usually bovine serum albumin or bovine γ-globulin) to create a standard curve from which the concentration of the sample is derived (for a protocol describing protein quantitation, refer to Part II of this guide).

Protein Assays

The chemical components of the sample buffer and the amount of protein available for assay dictate the type of assay that may be used (Table 3.2).

- Bradford assays (Bradford 1976) — are based on an absorbance shift of Coomassie (Brilliant) Blue G-250 dye under acid conditions. A redder form of the dye is converted into a bluer form upon binding to protein. The increase of absorbance at 595 nm is

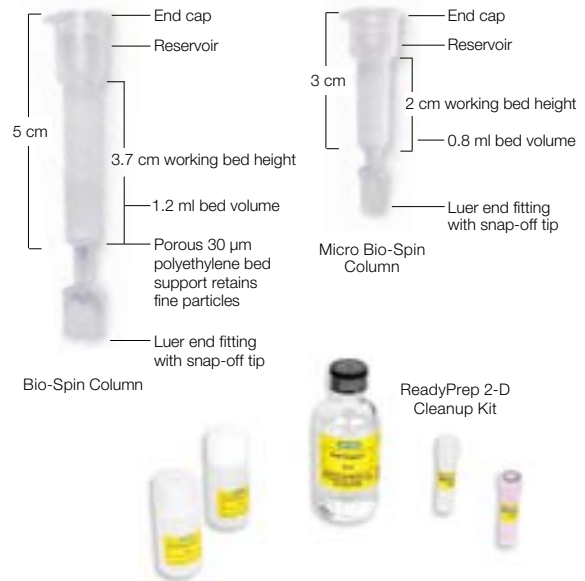


Fig. 3.4. Bio-Rad products that can be used for contaminant removal. Top, Micro Bio-Spin and Bio-Spin columns; Bottom, ReadyPrep 2-D cleanup kit.

Table 3.2. Bio-Rad protein assay selection guide.

| | Quick Start™ Bradford | Bio-Rad | DC™ | RC DC™ |
|------------------------------|---|--|--|---|
| Method | • | • | — | — |
| Bradford | — | — | • | • |
| Lowry | — | — | — | • |
| Description | One-step determination; not to be used with high levels of detergents (>0.025% SDS) | Standard Bradford assay, not to be used with elevated levels of detergents (>0.1% SDS) | Detergent compatible (DC); Lowry assay modified to save time | Reducing agent detergent compatible (RC DC) |
| Standard-Concentration Assay | | | | |
| Sample volume | 100 µl | 100 µl | 100 µl | 100 µl |
| Linear range | 0.125–1.5 mg/ml | 0.125–1.5 mg/ml | 0.125–1.5 mg/ml | 0.2–1.5 mg/ml |
| Low-Concentration Assay | | | | |
| Sample volume | 1 ml | 800 µl | 200 µl | 200 µl |
| Linear range | 1.25–25 µg/ml | 1.25–25 µg/ml | 5–250 µg/ml | 5–250 µg/ml |
| Microplate assay volume | 5 µl | 10 µl | 5 µl | ** |
| Minimum incubation | 5 min | 5 min | 15 min | 15 min |
| Assay wavelength | 595 nm | 595 nm | 650–750 nm | 650–750 nm |

proportional to the amount of bound dye, and thus to the amount (concentration) of protein in the sample. Compared with other protein assays, the Bradford protein assay is less susceptible to interference by various chemicals that may be present in protein samples*

- Lowry (Lowry et al. 1951) — combines the reactions of cupric ions with peptide bonds under alkaline conditions and the oxidation of aromatic protein residues. The Lowry method is based on the reaction of Cu⁺, produced by the peptide-mediated reduction of Cu²⁺, with Folin-Ciocalteu reagent (a mixture of phosphotungstic acid and phosphomolybdic acid in the Folin-Ciocalteu reaction)
- BCA (bicinchoninic acid, Smith et al. 1985) — reacts directly with Cu⁺ (generated by peptide-mediated reduction of Cu²⁺) to produce a purple end product. The reagent is fairly stable under alkaline conditions and can be included in the copper solution to make the assay a one-step procedure

To measure protein concentration in Laemmli buffers, use the reducing agent detergent compatible (RC DC™) protein assay, which is compatible with reducing agents and detergents. For more information on protein quantitation using colorimetric assays, refer to Bio-Rad bulletin 1069.

*The Bradford assay is, however, highly sensitive to ionic detergents like SDS.

SmartSpec™ Plus Spectrophotometer

The color change observed in protein assays is monitored and quantitated by a spectrophotometer. Bio-Rad's SmartSpec Plus spectrophotometer (Figure 3.5) has preprogrammed methods for protein quantitation and a working wavelength range of 200–800 nm. It can be used for routine applications such as:

- Quantitation of proteins via the Bradford, Lowry, and BCA assay methods
- Quantitation of DNA, RNA, and oligonucleotides
- Monitoring bacterial culture growth
- Simple kinetic assays
- Wavelength scans with peak detection



Fig. 3.5. SmartSpec Plus spectrophotometer.

Features built into the SmartSpec assay methods facilitate data collection and present a complete analysis of assay results. Bio-Rad also offers compatible quartz and UV-transparent plastic cuvettes.



CHAPTER 4

Reagent Selection and Preparation

This chapter details how to select and prepare the reagents (protein standards, gels, and buffers) required for various PAGE applications. The types of gels and buffers selected should suit the size of the protein under investigation, the desired analysis technique, and the overall goals of the experiment.

Links

Recombinant Protein Standards (Markers)

Precision Plus Protein Unstained Standards

Precision Plus Protein Prestained Standards

Precision Plus Protein All Blue Standards

Precision Plus Protein Dual Color Standards

Precision Plus Protein Dual Xtra Standards

Precision Plus Protein Kaleidoscope Standards

Precision Plus Protein WesternC Standards

Precision Protein StrepTactin-HRP Conjugate

General Considerations

No particular gel type or buffer is useful for all proteins, and choosing the buffer systems and gel types that offer the highest resolution in the size range of interest may require some experimentation. In selecting reagents for PAGE, consider the following:

- Protein standards — select protein standards that provide maximum resolution in the size range of interest and that offer compatibility and utility for downstream applications such as western blotting
- Gel percentage — choose the percentage that offers the best resolution in the range of interest
- Handcast vs. precast gels — precast gels offer greater convenience and superior quality control and reproducibility than handcast gels; handcast gels provide customized percentages and gradients
- Gel format — select mini- or midi-format gels when throughput is important or sample size is limited; select large-format gels for higher resolution. Select a comb type and gel thickness to accommodate the sample number and volume you are working with
- Buffer system — choose the system that offers the best resolution and compatibility with the protein and application of interest

Protein Standards

Protein standards are mixtures of well-characterized or recombinant proteins that are loaded alongside protein samples in a gel. They are used to monitor separation as well as estimate the size and concentration of the proteins separated in a gel.

Table 4.1. Applications of Bio-Rad's protein standards.

| | Precision Plus Protein™ Standards | | | | | | Prestained Natural Standards | | | |
|--------------------------------|-----------------------------------|---------------|-----------|----------|-----------|-----------|------------------------------|-----------|-------------|----------------------|
| | Dual Color | Kaleidoscope™ | Dual Xtra | All Blue | WesternC™ | Unstained | High Range | Low Range | Broad Range | Natural Kaleidoscope |
| Electrophoresis | | | | | | | | | | |
| Accurate MW estimation | • | • | • | • | • | • | — | — | — | — |
| Visualize electrophoresis | • | • | • | • | • | — | • | • | • | • |
| Orientation | • | • | • | — | • | — | — | — | — | — |
| Extended MW range | — | — | • | — | — | — | — | — | — | — |
| Coomassie staining | • | • | • | • | • | • | • | • | • | • |
| Fluorescent staining | — | — | — | — | — | • | — | — | — | — |
| Blotting | | | | | | | | | | |
| Monitoring transfer efficiency | • | • | • | • | • | — | • | • | • | • |
| Coomassie staining | • | • | • | • | • | • | • | • | • | • |
| Immunodetection | — | — | — | — | • | • | — | — | — | — |
| Fluorescent blots* | • | • | • | • | • | — | — | — | — | — |

MW = molecular weight.
* For use with fluorescent blots, not to be confused with fluorescent total blot stains. Precision Plus Protein prestained standards contain dyes with fluorescent properties. See bulletin 5723 for details on using precision Plus Protein WesternC standards for fluorescent multiplexing.

Select protein standards that offer:

- Good resolution of the proteins in the size range of interest
- Compatibility with downstream analysis (for example, blotting)

Protein standards are available as prestained or unstained sets of purified or recombinant proteins. In general, prestained standards allow easy and direct visualization of their separation during electrophoresis and their subsequent transfer to membranes. Although prestained standards can be used for size estimation, unstained protein standards will provide the most accurate size determinations.

Applications and details of Bio-Rad's protein standards are provided in Table 4.1.

Recombinant Standards

Recombinant standards are engineered to display specific attributes such as evenly spaced molecular weights or affinity tags for easy detection. Bio-Rad's recombinant standards are the Precision Plus Protein standards family and are available as stained or unstained standards (Figure 4.1). These standards contain highly purified recombinant proteins with molecular masses of 10–250 kD (or 2–250 kD for the Dual Xtra standards).

- Precision Plus Protein unstained standards — include three high-intensity reference bands (25, 50, and 75 kD) and contain a unique affinity *Strep*-tag, which allows detection and molecular weight determination on western blots. These standards offer absolute molecular weight accuracy confirmed by mass spectrometry. Because

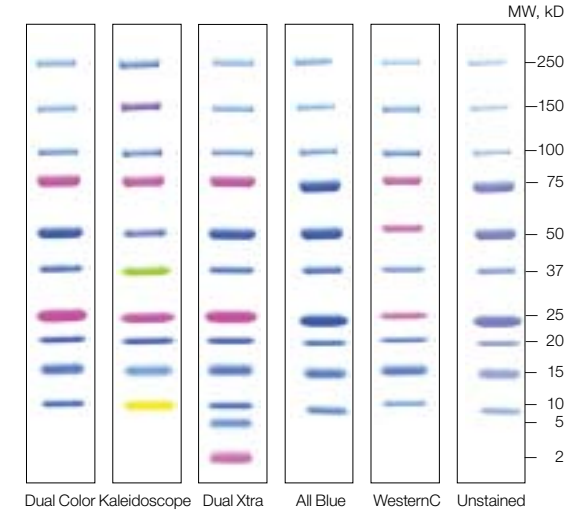


Fig. 4.1. Precision Plus Protein family of protein standards.

they contain a known amount of protein in each band, they also allow approximation of protein concentration. These standards are compatible with Laemmli and neutral pH buffer systems and are an excellent choice for use with Stain-Free™ technology (since they do not contain dye that can interfere with stain-free detection). See Stain-Free Technology box in Chapter 6 for more details

- Precision Plus Protein prestained standards (All Blue, Dual Color, and Kaleidoscope) — include a proprietary staining technology that provides batch-to-batch molecular mass consistency and reproducible migration. The ability to visualize these standards makes them ideal for monitoring protein separation during gel electrophoresis
- Precision Plus Protein Dual Xtra standards — prestained standards with additional 2 and 5 kD bands to enable molecular mass estimation below 10 kD
- Precision Plus Protein™ WesternC™ standards — dual color, prestained, and broad range protein standards that enable chemiluminescence detection when probed with StrepTactin-HRP conjugates; the protein standard appears directly on a film or CCD image. Additionally this protein standard has fluorescent properties that enable detection for fluorescent blots*

Natural Standards

Natural standards are blended from naturally occurring proteins. Although prestained natural standards are effective for monitoring gel separation and efficiency, they covalently bind the dye in different amounts and at different locations. This may produce broader bands than are seen with recombinant standards, making them less desirable for molecular weight estimations.

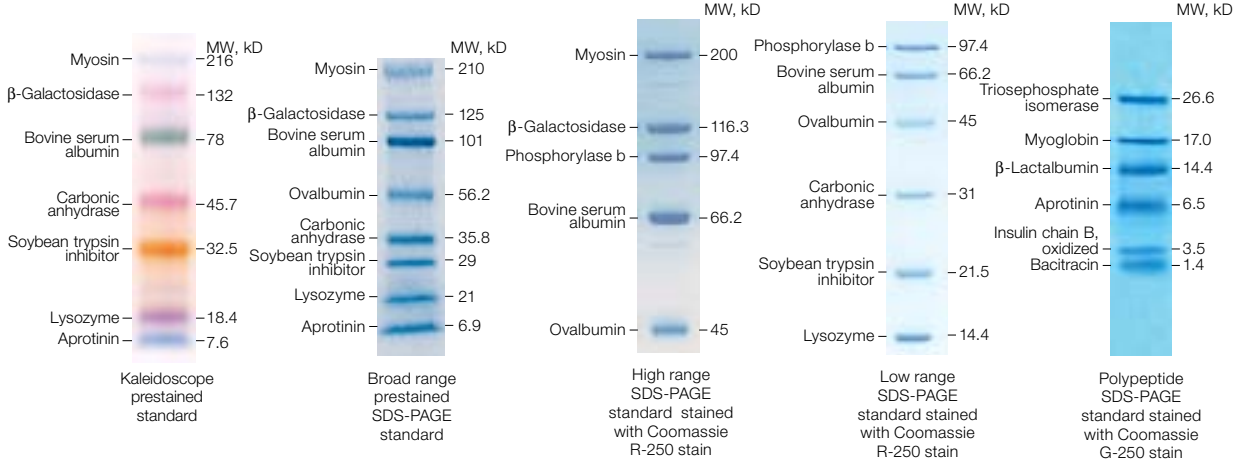


Fig. 4.2. Bio-Rad's natural protein standards.

*Prestained standards are not recommended for use on gels that will be stained with fluorescent dyes.

Bio-Rad offers the following prestained and unstained natural standards (Figure 4.2):

- Kaleidoscope standards — individually colored proteins to allow instant orientation on gels and blots
- Prestained SDS-PAGE standards — blue colored standards available in low, high, and broad ranges
- Unstained SDS-PAGE standards — available in low, high, and broad ranges. These standards are blended to give uniform band intensities when stained with Coomassie (Brilliant) Blue R-250 or zinc stains
- Polypeptide SDS-PAGE standards — for molecular weight determination of peptides and small proteins resolved on Tricine gels

Polyacrylamide Gels

Polyacrylamide is stable, chemically inert, electrically neutral, hydrophilic, and transparent for optical detection at wavelengths greater than 250 nm. These characteristics make polyacrylamide ideal for protein separations because the matrix does not interact with the solutes and has a low affinity for common protein stains (Garfin 2009).

Polymerization

Polyacrylamide gels are prepared by free radical polymerization of acylamide and a comonomer cross-linker such as bis-acrylamide. Polymerization is initiated by ammonium persulfate (APS) with tetramethylethylenediamine (TEMED) acting as a catalyst (Figure 4.3). Riboflavin (or riboflavin-5'-phosphate) may also be used as a source of free radicals, often in combination with TEMED and APS. Polymerization speed depends on various factors (monomer and catalyst concentration, temperature,

Related Literature

- Acrylamide Polymerization — A Practical Approach, bulletin 1156
- The Little Book of Standards, bulletin 2414
- Protein Standards Application Guide, bulletin 2998
- Increase Western Blot Throughput with Multiplex Fluorescent Detection, bulletin 5723
- Precision Plus Protein Dual Xtra Standards—New Protein Standards with an Extended Range from 2 to 250 kD, bulletin 5956

Links

Natural Standards

Prestained SDS-PAGE Standards

Kaleidoscope Prestained Standards

High Range Prestained SDS-PAGE Standards

Low Range Prestained SDS-PAGE Standards

Broad Range Prestained SDS-PAGE Standards

Unstained SDS-PAGE Standards

High Range Unstained SDS-PAGE Standards

Low Range Unstained SDS-PAGE Standards

Broad Range Unstained SDS-PAGE Standards

Polypeptide SDS-PAGE Standards

Coomassie Stains

Coomassie Brilliant Blue R-250 Stain

Coomassie Brilliant Blue G-250 Stain

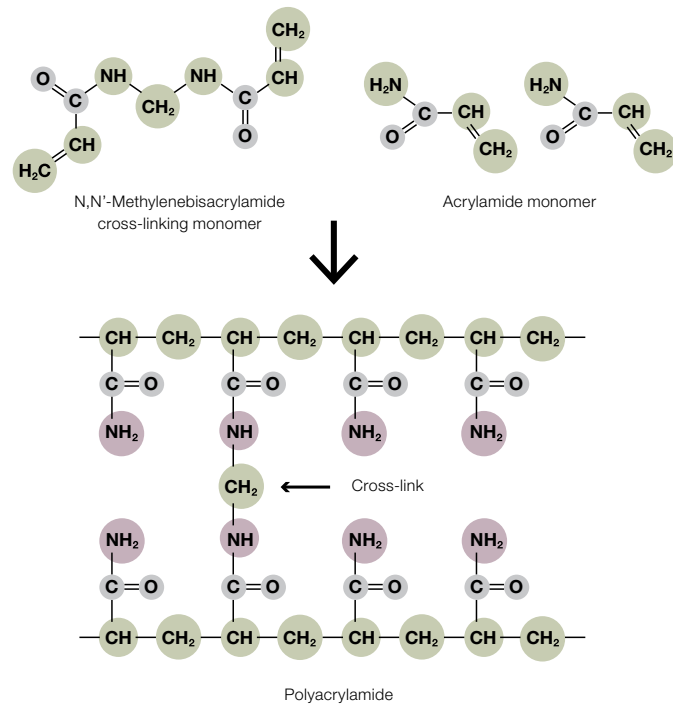


Fig. 4.3. Polymerization of acrylamide monomers and bisacrylamide.

and purity of reagents) and must be carefully controlled because it generates heat and may lead to nonuniform pore structures if it is too rapid.

Percentage

Polyacrylamide gels are characterized by two parameters: total monomer concentration (%T, in g/100 ml) and weight percentage of cross-linker (%C). By varying these two parameters, the pore size of the gel can be optimized to yield the best separation and resolution for the proteins of interest. %T indicates the relative pore size of the resulting polyacrylamide gel; a higher %T refers to a larger polymer-to-water ratio and smaller average pore sizes.

$$\%T = \frac{\text{g acrylamide} + \text{g cross-linker}}{\text{Total volume, ml}} \times 100$$

$$\%C = \frac{\text{g cross-linker}}{\text{g acrylamide} + \text{g cross-linker}} \times 100$$

The practical ranges for monomer concentration are stock solutions of 30–40%, with different ratios of acrylamide monomer to cross-linker. The designations 19:1, 29:1, or 37.5:1 on acrylamide/bis solutions represent cross-linker ratios of 5%, 3.3%, and 2.7% (the most common cross-linker concentrations for protein separations).

Gels can be made with a single, continuous percentage throughout the gel (single-percentage gels), or they can be cast with a gradient of %T through the gel (gradient gels). Typical gel compositions are between 7.5% and 20% for single-percentage gels, and typical gradients are 4–15% and 10–20%. Use protein migration charts and tables to select the gel type that offers optimum resolution of your sample (Figure 4.4):

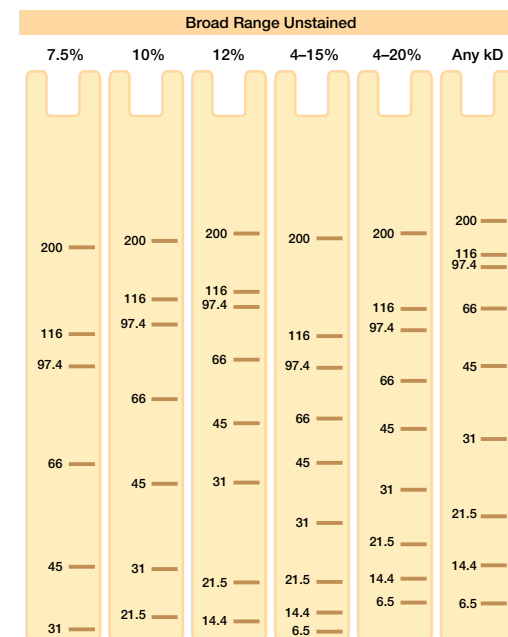
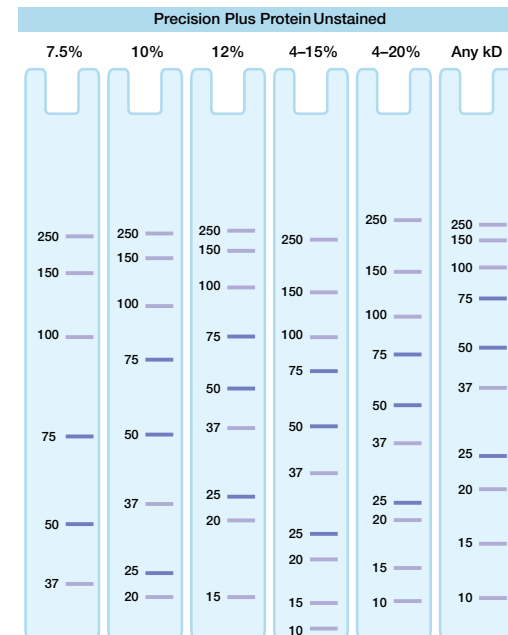


Fig. 4.4. Examples of migration charts.

- Use single-percentage gels to separate bands that are close in molecular weight. Since optimum separation occurs in the lower half of the gel, choose a percentage in which your protein of interest migrates to the lower half of the gel
- Use gradient gels to separate samples containing a broad range of molecular weights. Gradient gels allow resolution of both high- and low-molecular weight bands on the same gel. The larger pore size toward the top of the gel permits resolution of larger molecules, while pore sizes that decrease toward the bottom of the gel restrict excessive separation of small molecules
- For new or unknown samples, use a broad gradient, such as 4–20% or 8–16%, for a global evaluation of the sample. Then move to using an appropriate single-percentage gel once a particular size range of proteins has been identified

Precast vs. Handcast

Precast gels are ready to use and offer greater convenience, more stringent quality control, and higher reproducibility than handcast gels. Many precast gels also provide a shelf life of up to 12 months, allowing gels to be stored and used as needed (this is not possible with handcast gels, as they degrade within a few days).

Handcast gels, on the other hand, must be prepared from acrylamide and bisacrylamide monomer solutions; the component solutions are prepared, mixed together, and then poured between two glass plates to polymerize (see Part II of this guide for a detailed protocol). Because acrylamide and bisacrylamide are neurotoxins when in solution, care must be taken to avoid direct contact with the solutions and to clean up any spills. In addition, the casting process requires hours to complete, is not as controlled as it is by gel manufacturers, and contributes to more irregularities and less reproducibility with handcast gels.

Although handcasting offers the benefit of customized percentages, chemistries, and gradients, precast gels are sized to fit specific electrophoresis cells and are available in a range of chemistries, formulations, comb types, and thicknesses. Precast gels differ from their handcast counterparts in that they are cast with a single buffer throughout. Bio-Rad's precast gels (Table 4.3) also do not contain SDS and can be used for native or denaturing PAGE. For a complete and current list of available precast gels, visit the Bio-Rad website at **www.bio-rad.com**.

Format (Size and Comb Type)

The size format of the gel used depends on the electrophoresis cell selected (see Chapter 2). Precast gels are available for Bio-Rad's mini- and midi-format electrophoresis systems, and handcasting accessories are available to fit all Bio-Rad electrophoresis cells.

Additional parameters to consider include the number of wells and gel thickness, which depend on the number and volume of samples to analyze. To create sample wells in a gel, a comb is placed into the top of the gel prior to polymerization. When the comb is removed, a series of sample wells is left behind. The number and size of these wells dictate how many samples and what volume may be loaded (Table 4.2). The thickness of the gel also plays a role in determining the sample volume that can be loaded. A variety of comb types are available for handcasting; refer to www.bio-rad.com for more information.

Table 4.2. Comb types available for Bio-Rad precast polyacrylamide gels.

| | Comb Thickness, 1.0 mm | |
|--|------------------------|-----------------|
| | Number of Wells | Well Volume |
| Mini-Format Gels (Ready Gel® and Mini-PROTEAN®) | 8+1 | 30 µl |
| | 10 | 30 µl and 50 µl |
| | 12 | 20 µl |
| | 15 | 15 µl |
| | IPG | 7 cm IPG strip |
| Midi-Format Gels (Criterion™) | 12+2 | 45 µl |
| | 18 | 30 µl |
| | 26 | 15 µl |
| | Prep+2 | 800 µl |
| | IPG+1 | 11 cm IPG strip |

Buffer Systems and Gel Chemistries

The pH and ionic composition of the buffer system determine the power requirements and heavily influence the separation characteristics of a polyacrylamide gel.

Buffer systems include the buffers used to:

- Cast the gel
- Prepare the sample (sample buffer)
- Fill the electrode reservoirs (running buffer)

Most common PAGE applications utilize discontinuous buffer systems (Niepmann 2007), where two ions differing in electrophoretic mobility form a moving boundary when a voltage is applied (see Chapter 2). Proteins have an intermediate mobility, making them stack, or concentrate, into a narrow zone at the beginning of electrophoresis. As that zone moves through the gel, the sieving effect of the gel matrix causes proteins of different molecular weights to move at different rates (see Figure 2.2). Varying the types of ions used in the buffers changes the separation characteristics and stability of the gel. Table 4.3 summarizes the various types of gel and buffer systems available.

Table 4.3. Gel and buffer chemistries for PAGE. For a current list of precast gels available from Bio-Rad, visit www.bio-rad.com.

| Gel Type | Selection Criteria | Buffers | | Precast (Format) Gels | | |
|----------------------|---|---------------|-----------------------------------|-----------------------|-----------|----------|
| | | Sample | Running | Mini-PROTEAN* | Criterion | Handcast |
| SDS-PAGE | | | | | | |
| Tris-HCl, pH 8.6 | Easy to prepare, reagents inexpensive and readily available; best choice when switching between precast and handcast gels and need to compare results | Laemmli | Tris/glycine/SDS | • (Ready Gel) | • | • |
| TGX™ | Laemmli-like extended shelf life gels; best choice when long shelf life is needed and traditional Laemmli separation patterns are desired | Laemmli | Tris/glycine/SDS | • (Mini-PROTEAN) | • | — |
| Stain-Free™ | Laemmli-like gels with trihalo compounds for rapid fluorescence detection without staining | Laemmli | Tris/glycine/SDS | — | • | — |
| TGX Stain-Free™ | Laemmli-like extended shelf life gels with trihalo compounds for rapid fluorescence detection without staining | Laemmli | Tris/glycine/SDS | • (Mini-PROTEAN) | • | — |
| Bis-Tris, pH 6.4 | Offer longest shelf life, but reagents may be costly | XT | XT MOPS or XT MES | — | • | — |
| Tris-acetate, pH 7.0 | Offer best resolution of high molecular weight proteins; useful in peptide sequencing or mass spectrometry applications | XT or Tricine | XT Tricine or Tris/Tricine/SDS | — | • | • |
| Native PAGE | | | | | | |
| Tris-HCl, pH 8.6 | Retention of native protein structure, resolution of proteins with similar molecular weight | Native | Tris/glycine | • (Ready Gel) | • | • |
| TGX | Laemmli-like extended shelf life gels; best choice when long shelf life is needed and traditional Laemmli separation patterns are desired | Native | Tris/glycine | • (Mini-PROTEAN) | • | — |
| Stain-Free | Laemmli-like gels with trihalo compounds for rapid fluorescence detection without staining | Native | Tris/glycine | — | • | — |
| TGX Stain-Free | Laemmli-like extended shelf life gels with trihalo compounds for rapid fluorescence detection without staining | Native | Tris/glycine | • (Mini-PROTEAN) | • | — |
| Tris-acetate, pH 7.0 | Offer best separation of high molecular weight proteins and protein complexes | Native | Tris/glycine | — | • | • |
| Peptide Analysis | | | | | | |
| Tris-Tricine | Optimized for separating peptide and proteins with molecular weight <1,000 | Tricine | Tris/Tricine/SDS | • (Mini-PROTEAN) | • | • |
| IEF | | | | | | |
| IEF | Cast with Bio-Lyte® ampholytes, allow separation by protein pI; contain no denaturing agents, so IEF is performed under native conditions | 50% glycerol | IEF cathode and IEF anode buffers | • (Ready Gel) | • | — |
| Protease Detection | | | | | | |
| Zymogram | Cast with gelatin or casein, which acts as a substrate for proteases that are separated in the gel under nondenaturing conditions | Zymogram | Tris/glycine/SDS | • (Ready Gel) | • | • |

* The Mini-PROTEAN gel running systems are compatible with both Ready Gels and Mini-PROTEAN gels. For gel types available in a Mini-PROTEAN compatible format, the table indicates whether they are available as Ready Gels or Mini-PROTEAN gels.

Laemmli (Tris-HCl)

The Laemmli system has been the standard system for SDS- and native PAGE applications for many years. Many researchers use Tris-HCl gels because the reagents are inexpensive and readily available; precast gels are also readily available in a wide variety of gel percentages.

This discontinuous buffer system relies on the stacking effect of a moving boundary formed between the leading ion (chloride) and the trailing ion (glycinate). Tris buffer is the common cation. Tris-HCl gels can be used in either denaturing SDS-PAGE mode (using Laemmli sample buffer and Tris/glycine/SDS running buffer) or in native PAGE mode (using native sample and running buffers without denaturants or SDS).

Tris-HCl resolving gels are prepared at pH 8.6–8.8. At this basic pH, polyacrylamide slowly hydrolyzes to polyacrylic acid, which can compromise separation. For this reason, Tris-HCl gels have a relatively short shelf life. In addition, the gel pH can rise to pH 9.5 during a run, causing proteins to undergo deamination and alkylation. This may diminish resolution and complicate postelectrophoresis analysis.

Bio-Rad has developed TGX™ (Tris-Glycine eXtended shelf life) precast gels, modified Laemmli gels with a proprietary modification that extends shelf life to 12 months and allows gels to be run at higher voltages without producing excess heat. The TGX formulation yields run times as short as 15 min and Laemmli-like separation patterns with exceptionally straight lanes and sharp bands. TGX gels offer excellent staining quality and transfer efficiency (with transfer times as short as 15 min for tank blotting and as short as 3 min with the Trans-Blot® Turbo™ system), and they do not require special, expensive buffers. Like Tris-HCl gels, TGX gels use a discontinuous buffer system, with glycinate as the trailing ion, and are, therefore, compatible with conventional Laemmli and Tris/glycine/SDS buffers. These are the best choice when long shelf life is needed and traditional Laemmli separation patterns are desired.

Bio-RAD'S TGX Stain-Free™ gels are Laemmli-like, extended shelf life gels that allow rapid fluorescent detection of proteins with the Gel Doc™ EZ or ChemiDoc™ MP imaging systems, eliminating staining/destaining steps for completion of protein separation, visualization, and analysis in 25 min (see Stain-Free™ Technology box in Chapter 6 for more details).

Bis-Tris

These systems employ chloride as the leading ion and MES or MOPS as the trailing ion. The common cation is formed from Bis-Tris buffer. The gels are prepared at pH 6.4 to enhance gel stability. Running the same Bis-Tris gels with either MES or MOPS denaturing

running buffer produces different migration patterns: MES buffer is used for small proteins, and MOPS buffer is used for mid-sized proteins.

Precast Bis-Tris gels (for example, Criterion™ XT Bis-Tris gels) offer extended shelf life (compared to Tris-HCl gels) and room temperature storage. These gels are popular because of their stability but they require special buffers, and the gel patterns cannot be compared to those of Tris-HCl gels.

Common reducing agents such as βME and DTT are not ionized at the relatively low pH of Bis-Tris gels and so do not enter the gel and migrate with the proteins. Alternative reducing agents are, therefore, used with Bis-Tris gels to maintain a reducing environment and prevent protein reoxidation during electrophoresis.

Tris-Acetate

This discontinuous buffer system uses acetate as the leading ion and Tricine as the trailing ion and is ideally suited for SDS-PAGE of large proteins. Tris-acetate gels can be used for both SDS- and native PAGE. Like Bis-Tris gels, they offer extended shelf life and room temperature storage. Because of their lower pH, these gels offer better stability than Tris-HCl gels and are best suited for peptide sequencing and mass spectrometry applications.

Tris-Tricine

One of the drawbacks to using SDS in a separation system is that excess SDS runs as a large front at the low molecular weight end of the separation. Smaller polypeptides do not separate from this front and, therefore, do not resolve into discrete bands. Replacing the glycine in the Laemmli running buffer with Tricine yields a system that separates the small SDS-polypeptides from the broad band of SDS micelles that forms behind the leading-ion front. Proteins as small as 1–5 kD can be separated in these gels.

IEF

Isoelectric focusing (IEF) separates proteins by their net charge rather than molecular weight. IEF gels are cast with ampholytes, amphoteric molecules that generate a pH gradient across the gels. Proteins migrate to their pI, the pH at which the protein has no net charge. Since IEF gels contain no denaturing agents, IEF is performed under native conditions.

Zymogram

Zymogram gels contain either gelatin or casein, which are substrates for various proteases. Samples are run under denaturing but nonreducing conditions and are then allowed to renature and consume the substrate. Samples with proteolytic activity can be visualized as clear bands against a blue background after using Coomassie (Brilliant) Blue R-250 stain.

Related Literature

Mini-PROTEAN TGX Precast Gels Product Information Sheet, bulletin 5871

Mini-PROTEAN TGX Precast Gel: A Gel for SDS-PAGE with Improved Stability — Comparison with Standard Laemmli Gels, bulletin 5910

Mini-PROTEAN TGX Precast Gel: A Versatile and Robust Laemmli-Like Precast Gel for SDS-PAGE, bulletin 5911

Ready Gel to Mini-PROTEAN TGX Precast Gels Catalog Number Conversion Chart, bulletin 5932

NuPAGE Bis-Tris Precast Gels (MOPS Buffer) to Mini-PROTEAN TGX Precast Gels Catalog Number Conversion Chart, bulletin 5934

Criterion XT Precast Gels Product Information Sheet, bulletin 2911

Criterion TGX Stain-Free Precast Gels Product Information Sheet, bulletin 5974

Links

Mini Format 1-D Electrophoresis Systems

Mini-PROTEAN TGX Precast Gels

Midi Format 1-D Electrophoresis Systems

Criterion TGX Stain-Free Precast Gels

Criterion XT Bis-Tris Precast Gels

Trans-Blot Turbo Transfer System

Imaging Systems

ChemiDoc MP System

Gel Doc EZ Imaging System

Coomassie Stains

Coomassie Brilliant Blue R-250 Stain

Coomassie Brilliant Blue G-250 Stain

Sample Buffers and Reagents

Running Buffers and Reagents

Related Literature

Ready-to-Run Buffers and Solutions Brochure, bulletin 2317

Products for Handcasting Gels

The following products are available to facilitate handcasting gels. For detailed handcasting protocols, refer to Part II of this guide.

Premade Buffers and Reagents

Electrophoresis buffers and reagents are available as individual reagents or as premixed gel-casting, sample, and running buffers. Use of commercially prepared, premixed buffers, which are made with electrophoresis-purity reagents and are quality controlled for reproducible results, saves time but also maximizes reproducibility, prevents potential mistakes in buffer concentration, and standardizes electrophoresis runs. There are no reagents to weigh or filter; just dilute with distilled or deionized water.

AnyGel™ Stands

AnyGel stands (Figure 4.5) provide stabilization and access to gels for casting and sample loading. The clamping mechanism secures gel cassettes vertically without excess pressure. They are available in two sizes, single- and six-row.



Fig. 4.5. AnyGel stands.

Multi-Casting Chambers

Multi-casting chambers are used to cast multiple gels of various thicknesses simultaneously. Acrylic blocks act as space fillers when fewer than the maximum number of gels are cast. These chambers work in concert with the gradient formers through a bottom filling port to ensure reproducibility. Multi-casting chambers are available for casting gels for the Mini-PROTEAN, PROTEAN® II, and PROTEAN Plus systems.

Gradient Formers

Gradient gels have a gradient of acrylamide concentration that increases from top to bottom. To create this gradient, the acrylamide solutions must be

mixed in a gradient former before being introduced into the gel cassette. Typically, two solutions are prepared: the light solution (equivalent to the lowest %T in the range to be poured) and a heavy solution (equivalent to the maximum %T to be poured). The most common gradient gel contains 4–20% acrylamide; however, the range of acrylamide concentrations should be chosen on the basis of the size of the proteins being separated.

Two gradient formers are available for PAGE systems. Depending on the gel format, prepare either a single gel using the gradient former or couple the gradient former with a multi-casting chamber to prepare up to 12 gels simultaneously (Figure 4.6).

- Use the Model 485 gradient former to cast a minimum of 4 mini-format gels at a time using the Mini-PROTEAN 3 multi-casting chamber, or to cast a single, large-format (PROTEAN II or PROTEAN Plus) gel
- Use the Model 495 gradient former to prepare 4–12 large-format gels (PROTEAN II and PROTEAN Plus) using the multi-gel casting chambers



Fig. 4.6. Multi-casting chambers and gradient formers.

Links

- AnyGel Stand
- Polyacrylamide Gel Reagents
- Premixed Casting Buffers
- Mini-PROTEAN Tetra Handcast Systems
- PROTEAN Plus Multi-Casting Chamber
- PROTEAN Plus Hinged Spacer Plates and Combs
- Model 485 Gradient Former
- Model 495 Gradient Former



CHAPTER 5

Performing Electrophoresis

In this phase of the workflow, the electrophoresis system is assembled, samples are loaded, and electrophoresis is initiated by programming the power supply. Select running conditions that provide optimum resolution while maintaining the temperature of the system during separation.

System Setup

System setup involves placing the gels in the tank, filling the tank with running buffer, loading the samples and protein standards, and programming the power supply. Follow the instructions for system setup in the instruction manuals for the system you are using. General procedures and tips are provided in Part II of this guide.

Running Conditions

Regulated direct current (DC) power supplies allow control over every electrophoresis mode (constant voltage, current, or power). The choice of which electrical parameter to control is usually a matter of preference.

Useful Equations

In PAGE separations, the gel containing the protein sample is placed in the electrophoresis chamber between two electrodes. The driving force behind the separation is the voltage (V, in volts) applied across the electrodes. This leads to a current flow (I, in amperes) through the gel, which has an intrinsic resistance (R, in ohms). Ohm’s law describes the mutual dependence of these three parameters:

$I = V/R \text{ or } V = IR \text{ or } R = V/I$

The applied voltage and current are determined by the user and the power supply settings; the resistance is inherent in the system and is determined by the ionic strength of the buffer, the conductivity of the gel, and other factors.

The power (P, in watts) consumed by an electrical current element is equal to the product of the voltage and current:

$P = VI = I^2R = V^2/R$

The strength of the electric field E (V/cm) applied between the two electrodes is an important parameter in electrophoresis, because it exerts a force on electrically charged objects like proteins and determines their migration rate (where d is the distance in cm):

$E = V/d$

Most vertical electrophoresis chambers are operated at a field strength of 10–20 V/cm for 1 mm thick polyacrylamide gels.

Joule Heating

The electric field strength (E, in V/cm) that can be generated between the electrodes is limited by the heat that is inevitably produced during electrophoresis. This Joule heating can lead to band distortion, increased diffusion, and protein denaturation when not efficiently removed from the system. The amount of Joule heating

that occurs depends on the conductivity of the buffer used, the magnitude of the applied field, and the total resistance within the system.

The heat generated is proportional to the power consumed (P):

$Heat = P/4.18 \text{ cal/sec}$

Understanding the relationships between power, voltage, current, resistance, and heat is central to understanding the factors that influence the efficiency and efficacy of electrophoresis. The optimum condition is to run at the highest electric field strength possible within the heat dissipation capabilities of the system.

During an electrophoretic separation using the Ornstein-Davis and Laemmli systems, the running buffer warms as a result of Joule heating. The increase in temperature may lead to inconsistent field strength and separation and may cause the buffer to lose its buffering capacity or the gel to melt or become distorted. Under normal running conditions, the running buffer absorbs most of the heat that is generated. However, during extended runs or high-power conditions, active buffer cooling is required to prevent uncontrolled temperature increases.

Other Factors Affecting Electrophoresis

The following variables also change the resistance of the system and, therefore, affect separation efficiency and current and voltage readings:

- Alterations to buffer composition; that is, the addition of SDS or changes in ion concentration due to the addition of acid or base to adjust the pH of a buffer
- Gel pH, ionic strength, and percentage of acrylamide
- Number of gels (current increases as the number of gels increases)
- Volume of buffer (current increases when volume increases)
- Transfer temperature (current increases when temperature increases)
- Gel length (increasing gel length demands higher voltage settings to increase field strength accordingly)
- Gel thickness (increasing gel width or thickness at identical gel length leads to higher current; voltage must be kept unchanged)

Selecting Power Supply Settings

Power supplies that are used for electrophoresis hold one parameter constant (either voltage, current, or power). The PowerPac™ HC and PowerPac Universal power supplies also have an automatic crossover capability that allows the power supply to switch over to a variable parameter if a set output limit is reached. This prevents damage to the electrophoresis cell.

The resistance, however, does not remain constant during a run:

- In continuous buffer systems (for example, those used for blotting or DNA separation), resistance declines with increasing temperature caused by Joule heating
- In discontinuous systems, such as the Ornstein-Davis (native) and Laemmli (SDS-PAGE) systems, resistance also changes as discontinuous buffer ion fronts move through the gel; in SDS-PAGE, resistance increases as the run progresses. Depending on the buffer and which electrical parameter is held constant, Joule heating of the gel may increase or decrease over the period of the run

Separations Under Constant Voltage

If the voltage is held constant throughout a separation, the current and power (heat) decrease as the resistance increases. This leads to increased run times, which allow the proteins more time to diffuse. But this appears to be offset by the temperature-dependent increase in diffusion rate of the constant current mode. Separations using constant voltage are often preferred because a single voltage that is independent of the number of gels being run is specified for each gel type.

Separations Under Constant Current

If the current is held constant during a run, the voltage, power, and consequently the heat of the gel chamber increase during the run. As a rule, constant current conditions result in shorter but hotter runs than constant voltage runs.

Separations Under Constant Power

Holding the power constant minimizes the risk of overheating.

General Guidelines for Running Conditions

Electrophoresis cells require different power settings with different buffer systems. The values presented are guidelines — conditions should be optimized for each application. In every case, run the gel until the dye front reaches the bottom of the gel.

Use external cooling during long, unsupervised runs. Temperature-controlled runs often yield more uniform and reproducible results.

For best results:

- Increase run times for gradient gels and decrease them as needed for low molecular weight proteins
- For the first ~10 min of a run, allow the sample to stack using a field strength of 5–10 V/cm gel length. Then continue with the maximum voltage recommended in the instruction manual of the electrophoresis system
- If using multiple cells and constant voltage, use the same voltage for multiple cells as you would for one cell. The current drawn by the power supply doubles with two — compared to one — cells. Set the current limit high enough to permit this additive function. Also be sure to use a power supply that can accommodate this additive current

Gel Disassembly and Storage

Remove the gel cassette and open it according to the manufacturer’s instructions. Before handling the gel, wet your gloves with water or buffer to keep the gel from sticking and to minimize the risk of tearing. Sometimes it is also helpful to lift one edge of the gel with a spatula.

Stain, blot, or process the gel as soon as possible to maintain the resolution achieved during electrophoresis and to keep the gel from drying out (see Chapters 6 and 7). For long-term storage, either dry stained gels in a 10% glycerol solution (storage at 4°C) or with the GelAir™ drying system, which allows gels to dry between two sheets of cellophane within 60 min. This yields clear, publication-quality gels ideal for densitometry.

Links

Power Supplies

PowerPac HC High-Current Power Supply

PowerPac Universal Power Supply

GelAir Drying System



CHAPTER 6

Protein Detection and Analysis

Following electrophoresis, protein band patterns can be visualized and subjected to qualitative and quantitative analysis. Since most proteins cannot be seen in a gel with the naked eye, protein visualization is usually achieved through use of protein stains. Once the gel is stained, it can be imaged and analyzed using imaging instruments and accompanying software.

Related Literature

Rapid Validation of Purified Proteins Using Criterion Stain Free Gels, bulletin 6001

Sensitivity and Protein-to-Protein Consistency of Flamingo Fluorescent Gel Stain Compared to Other Fluorescent Stains, bulletin 5705

Comparison of SYPRO Ruby and Flamingo Fluorescent Gel Stains With Respect to Compatibility With Mass Spectrometry, bulletin 5754

Oriole Fluorescent Gel Stain: Characterization and Comparison with SYPRO Ruby Gel Stain, bulletin 5921

Bio-Safe Coomassie Stain Brochure, bulletin 2423

Flamingo Fluorescent Gel Stain Product Information Sheet, bulletin 5346

Links

Criterion Precast Gels

Criterion TGX Stain-Free Precast Gels

Mini-PROTEAN TGX Stain-Free Gels

Coomassie Stains

QC Colloidal Coomassie Stain

Bio-Safe Coomassie Stain

Coomassie Brilliant Blue R-250 Stain

Coomassie Brilliant Blue G-250 Stain

Fluorescent Protein Stains

Flamingo Fluorescent Gel Stain

Oriole Fluorescent Gel Stain

Silver Stains

Negative Stains

Protein Stains

In many cases, the choice of staining technique depends on the availability of imaging equipment. However, a protein staining technique should offer the following features (Miller et al. 2006):

- High sensitivity and reproducibility
- Wide linear dynamic range
- Compatibility with downstream technologies such as protein extraction and assay, blotting, or mass spectrometry
- Robust, fast, and uncomplicated protocol

Staining protocols usually involve the following three steps (protocols are available in Part II of this guide):

- Protein fixation, usually in acidic methanol or ethanol (a few staining protocols already contain acid or alcohols for protein fixation and so do not require this separate step)
- Exposure to dye solution
- Washing to remove excess dye (destaining)

Total Protein Stains

Total protein stains allow visualization of the protein separation pattern in the gel. Table 6.1 compares the advantages and disadvantages of several total protein staining techniques.

- Coomassie stains — the most popular anionic protein dye, Coomassie (Brilliant) Blue stains almost all proteins with good quantitative linearity at medium sensitivity. There are two variants of Coomassie (Brilliant) Blue: R-250 (R for reddish), which offers shorter staining times, and G-250 (G for greenish), which is available in more sensitive and environmentally friendly formulations (Simpson 2010, Neuhoﬀ et al. 1988). Coomassie dyes are also the favorite stains for mass spectrometry and protein identification. Bio-Rad oﬀers three variations of Coomassie Blue stains. The QC Colloidal Coomassie stain provides sensitivity down to ~3 ng BSA, low background endpoint staining, and reproducibility that is needed in regulated environments. Additionally, this colloidal stain is formulated to be ready-to-use (no additional prep or dilution needed) and to be environmentally friendly (no methanol or acetic acid waste disposal). Bio-Safe™ Coomassie stain is a nonhazardous formulation of Coomassie Blue G-250 that requires only water for rinsing and destaining. It oﬀers a sensitivity that is better than conventional Coomassie R-250 formulations and equivalent to Coomassie Blue G-250, but with a simpler and quicker staining protocol

- Fluorescent stains — fulfill almost all of the requirements for an ideal protein stain by oﬀering high sensitivity, a wide linear dynamic range over four orders of magnitude, a simple and robust protocol, and compatibility with mass spectrometry. In comparison to Coomassie or silver staining techniques, however, fluorescent dyes are more expensive and require either a CCD (charge-coupled device) camera or fluorescence scanner for gel imaging. For these reasons, fluorescent stains are often used in proteomics applications and on 2-D gels, where the relative quantitation of proteins in complex mixtures is performed over several orders of abundance and protein identity is determined using in-gel proteolytic digestion and mass spectrometry. Examples include Flamingo™ and Oriole™ fluorescent gel stains
- Silver stains — oﬀer the highest sensitivity, but with a low linear dynamic range (Merril et al. 1981, Rabilloud et al. 1994). Often, these protocols are time-consuming, complex, and do not oﬀer sufficient reproducibility for quantitative analysis. In addition, their compatibility with mass spectrometry for protein identification purposes is lower than that of Coomassie stains and fluorescent dyes (Yan et al. 2000)
- Negative stains — rapid negative stains require only ~15 min for high-sensitivity staining, where protein bands appear as clear areas in a white background. Zinc and copper stains do not require gel fixation, ensuring that proteins are not altered or denatured. Negative stains can be used as a quality check before transfer to a western blot or analysis by mass spectrometry, although they are not the best choice when quantitative information is desired
- Stain-free technology — a haloalkane compound in Bio-Rad’s Criterion™, Criterion™ TGX, and Mini-PROTEAN® TGX Stain-Free™ gels covalently binds to tryptophan residues of proteins when activated with UV light. This allows protein detection (with a stain-free compatible imager) in a gel both before and after transfer, as well as total protein detection on a blot when using PVDF membranes (see Stain-Free™ Technology box)

Specific Protein Stains

Specific protein stains are used to visualize specific protein classes such as glycoproteins (Hart et al. 2003) and phosphoproteins (Steinberg et al. 2003, Agrawal and Thelen 2009), which are of special interest to researchers working in the life sciences (examples include Pro-Q Diamond and Pro-Q Emerald).

Table 6.1. Bio-Rad Gel stain selection guide.

| Total Protein Stain | Sensitivity (Lower Limit) | Time | Comments | Detection Method |
|-----------------------------------|---------------------------|----------|--|------------------|
| Coomassie Stains | | | | Colorimetric |
| QC Colloidal Coomassie | 3 ng | 1–20 hr | Colloidal endpoint stain; premixed; nonhazardous formulation | |
| Bio-Safe Coomassie G-250 | 8–28 ng | 1–2.5 hr | Nonhazardous staining in aqueous solution; premixed, mass spectrometry compatible | |
| Coomassie Brilliant Blue R-250 | 36–47 ng | 2.5 hr | Simple and consistent; mass spectrometry compatible; requires destaining with methanol | Colorimetric |
| Silver Stains | | | | Colorimetric |
| Dodeca™ silver stain kit | 0.25–0.5 ng | 3 hr | Simple, robust; mass spectrometry compatible; ideal for use with Dodeca stainers (Sinha et al. 2001) | |
| Silver Stain Plus™ kit | 0.6–1.2 ng | 1.5 hr | Simple, robust; mass spectrometry compatible (Gottlieb and Chavko 1987) | |
| Silver stain (Merril et al. 1981) | 0.6–1.2 ng | 2 hr | Stains glycoproteins, lipoproteins, lipopolysaccharides, nucleic acids | Fluorescent |
| Fluorescent Stains | | | | Fluorescent |
| Oriole fluorescent gel stain* | 0.5–1 ng | 1.5 hr | Rapid protocol, requires no destaining, mass spectrometry compatible; compatible only with UV excitation | |
| Flamingo fluorescent gel stain | 0.25–0.5 ng | 5 hr | High sensitivity; broad dynamic range; simple protocol requires no destaining; mass spectrometry compatible; excellent for laser-based scanners | |
| SYPRO Ruby protein gel stain | 1–10 ng | 3 hr | Fluorescent protein stain; simple, robust protocol; broad dynamic range; mass spectrometry compatible | Colorimetric |
| Negative Stains | | | | Colorimetric |
| Zinc stain | 6–12 ng | 15 min | High-contrast results; simple, fast, and reversible; compatible with elution or blotting as well as mass spectrometry (Fernandez-Patron et al. 1992) | |
| Copper stain | 6–12 ng | 10 min | Single reagent; simple, fast, and reversible; compatible with elution or blotting as well as mass spectrometry (Lee et al. 1987) | |

* Do not use Oriole gel stain with native gels.

Stain-Free™ Technology

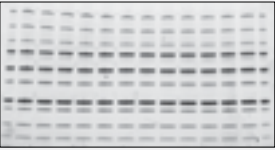
Bio-Rad’s stain-free technology allows direct visualization, analysis, and documentation of protein samples in PAGE gels without staining, destaining, or gel drying.

The system comprises the Gel Doc™ EZ and ChemiDoc™ MP imagers, Image Lab™ software, and precast gels that include unique trihalo compounds that allow rapid fluorescent detection of proteins — without staining. The trihalo compounds react with tryptophan residues in a UV-induced reaction to produce fluorescence, which can be detected by the imager either within gels or on low-fluorescence PVDF membranes. Activation of the trihalo compounds in the gels adds 58 Da moieties to available tryptophan residues and is required for protein visualization. Proteins that do not contain tryptophan residues are not detected.

The sensitivity of the Stain-Free system is comparable to staining with Coomassie (Brilliant) Blue for proteins with a tryptophan content >1.5%; sensitivity superior to Coomassie staining is possible for proteins with a tryptophan content >3%.

Benefits of stain-free technology include:

- Elimination of staining and destaining steps for faster results
- Automated gel imaging and analysis
- No background variability within a gel or between gels (as is often seen with standard Coomassie staining)
- Visualization of transferred (blotted) proteins on low-fluorescence PVDF membranes
- Reduced organic waste by eliminating the use of acetic acid and methanol in staining and destaining



Gel (top) and blot imaged using stain-free technology.

Links

SYPRO Ruby Protein Gel Stain

Dodeca Silver Stain Kit

Silver Stain Plus Kit

Zinc Stain

Copper Stain

Imaging Systems

ChemiDoc MP System

Gel Doc EZ System

Image Lab Software

Related Literature

Bio-Rad Imaging Systems Family Brochure, bulletin 5888

Imaging Fluorescently Stained Gels with Image Lab Software Quick Start Guide, bulletin 5989

Links

- High-Throughput Dodeca Gel Stainers
- Criterion Precast Gels
- Coomassie Stains
- Bio-Safe Coomassie Stain
- Coomassie Brilliant Blue G-250 Stain
- Coomassie Brilliant Blue R-250 Stain
- Fluorescent Protein Stains
- Flamingo Fluorescent Gel Stain
- Oriole Fluorescent Gel Stain
- SYPRO Ruby Protein Gel Stain
- Silver Stains
- Dodeca Silver Stain Kit
- Imaging Systems
- GS-900 Calibrated Densitometer
- Gel Doc EZ System
- PharosFX and PharosFX Plus Systems
- Gel Doc XR+ System

Dodeca™ High-Throughput Stainers

Dodeca stainers are high-throughput gel staining devices available in two sizes (Figure 6.1): the small size accommodates up to 24 Criterion gels while the large size can accommodate up to 12 large-format gels. The stainers ensure high-quality, consistent results and eliminate gel breakage from excess handling.

The stainers feature a shaking rack that holds staining trays at an angle to allow air bubbles to escape and ensure uniform gel staining by protecting gels from breaking. They are compatible with the following stains:

- Bio-Safe Coomassie (Brilliant) Blue G-250 stain
- Coomassie (Brilliant) Blue R-250 stain
- Flamingo Fluorescent gel stain
- SYPRO Ruby protein gel stain
- Oriole fluorescent gel stain
- Dodeca silver stain kits

Imaging

Though total protein stains yield visible band patterns, in modern laboratory environments, electrophoresis patterns (called electropherograms) are digitized by dedicated image acquisition devices and data are analyzed with sophisticated software. Once the gels are digitized, the raw data can be stored for further reference.

Imaging Systems

Selecting image acquisition devices for the digitization of electrophoresis gels depends on the staining technique used (see also Table 6.2):

- Flat-bed densitometers — based on high-performance document scanners that have been modified to make them suitable for accurate scientific measurement of optical density. Modifications include automatic calibration to traceable reference standards, mathematical correction of image non-









uniformity, and environmental sealing against liquid spills in the laboratory. Densitometers measure the absorbance (for gels stained with visible dyes) or reflectance (of blots developed with colorimetric reagents) of visible light. Bio-Rad's GS-900™ calibrated densitometer provides a calibrated linear dynamic range to a NIST-traceable standard up to 3.4 optical density (OD) units

- CCD (charge-coupled device) cameras — operate with either trans-illumination provided by light boxes (visible or UV) positioned underneath the gel or blot for imaging a variety of stains (Coomassie, silver, fluorescence) or epi-illumination detected using colorimetric or fluorescent techniques. Supercooled CCD cameras reduce image noise, allowing detection of faint luminescent signals. Bio-Rad's Gel Doc™ EZ system provides four application-specific trays: a UV tray (for ethidium bromide staining of DNA gels and fluorescence imaging), a white tray (for Coomassie, copper, silver, and zinc stains), a blue tray (for nondestructive nucleic acid imaging), and a stain-free tray for direct visualization, analysis, and documentation of protein samples in polyacrylamide gels without staining, destaining, or gel drying (see Stain-Free Technology box)
- Laser-based scanners — offer the highest sensitivity, resolution, and linear dynamic range. They are powerful image acquisition tools for electrophoresis gels and blots stained with fluorescent dyes like Flamingo fluorescent gel stain. Lasers can be matched to the excitation wavelengths of a multitude of fluorophores. Bio-Rad's PharosFX™ systems use multiple lasers, which enhance application flexibility and allow optimal excitation of single- or multicolor fluorescent samples to enable detection of almost any fluorescent dye or label



Fig. 6.1. High-throughput Dodeca gel stainers.

Table 6.2. Bio-Rad imaging system selection guide.

| |  |  |  |  |  |  |  |  |
|-----------------------------------|---|---|---|---|---|---|---|---|
| Application | ChemiDoc™ MP | Gel Doc EZ | GS-900 Densitometer | Gel Doc XR+ | ChemiDoc XRS+ | PharosFX | PharosFX Plus | Personal Molecular Imager™ |
| Nucleic Acid Detection | | | | | | | | |
| Ethidium bromide stain | 5 | 4 | — | 4 | 4 | 5 | 5 | — |
| SYBR® Green I stain | 5 | 4 | — | 4 | 4 | 5 | 5 | — |
| SYBR® Safe stain | 5 | 4 | — | 4 | 4 | 5 | 5 | — |
| Fast Blast™ DNA stain | 4 | 4 | 5 | 4 | 4 | — | — | — |
| Protein Detection, 1-D Gels | | | | | | | | |
| Stain-free gels | 5 | 5 | — | — | — | — | — | — |
| Coomassie Blue stain | 4 | 4 | 5 | 4 | 4 | 2 | 2 | — |
| Silver stain | 4 | 4 | 5 | 4 | 4 | 2 | 2 | — |
| SYPRO Ruby protein gel stain | 4 | 4 | — | 4 | 4 | 5 | 5 | — |
| Flamingo fluorescent gel stain | 4 | 3 | — | 4 | 4 | 5 | 5 | — |
| Oriole fluorescent gel stain | 5 | 4 | — | 5 | 5 | — | — | — |
| Protein Detection, 2-D Gels | | | | | | | | |
| Coomassie blue stain | 4 | 3 | 5 | 3 | 3 | 2 | 2 | — |
| Silver stain | 4 | 3 | 5 | 3 | 3 | 2 | 2 | — |
| SYPRO Ruby protein gel stain | 4 | 3 | — | 3 | 3 | 5 | 5 | — |
| Flamingo fluorescent gel stain | 4 | 2 | — | 3 | 3 | 5 | 5 | — |
| Oriole fluorescent gel stain | 5 | 3 | — | 3 | 3 | — | — | — |
| Pro-Q stain | 4 | 2 | — | 2 | 3 | 5 | 5 | — |
| Cy2, Cy3, Cy5 label | 4 | — | — | — | — | 5 | 5 | — |
| Blot Detection | | | | | | | | |
| Stain-free blots | 5 | 5 | — | — | — | — | — | — |
| Coomassie Blue stain | 4 | — | 5 | 4 | 4 | 2 | 2 | — |
| Silver stain | 5 | — | 5 | 4 | 4 | 2 | 2 | — |
| SYPRO Ruby protein blot stain* | 5 | — | — | — | — | 5 | 5 | — |
| Immun-Star™ chemiluminescence | 5 | — | — | — | 4 | — | — | — |
| Chemifluorescence* | 5 | — | — | 1 | 1 | 4 | 4 | — |
| Quantum dot* | 5 | 2 | — | 2 | 2 | 5 | 5 | — |
| Multiplex fluorescence | 5 | — | — | — | — | 5 | 5 | — |
| Micro- and Macroarray** Detection | | | | | | | | |
| Radiolabel | — | — | — | — | — | — | 5 | 5 |
| Fluorescence | 5 | — | — | 2 | 2 | 5 | 5 | — |
| Immun-Star chemiluminescence | 5 | — | — | — | 4 | — | — | — |
| Colony Counting | | | | | | | | |
| Colorimetric detection | 5 | — | — | 4 | 4 | 3 | 3 | — |
| Fluorescence detection | 5 | — | — | 4 | 4 | 5 | 5 | — |
| Isotopic Detection | | | | | | | | |
| Radiolabel | — | — | — | — | — | — | 5 | 5 |
| X-ray film | 4 | 4 | 5 | 4 | 4 | 3 | 3 | — |

— Not recommended; 1–5, recommendation level (5 = highest).

* Optimal with low fluorescence PVDF membrane.

** With spot diameters ≥400 µm.

Links

- ChemiDoc MP System
- ChemiDoc XRS+ System
- Personal Molecular Imager System

Related Literature

- Molecular Weight Determination by SDS-PAGE, bulletin 3133
- Using Precision Plus Protein Standards to Determine Molecular Weight, bulletin 3144
- Molecular Weight Estimation Using Precision Plus Protein WesternC Standards on Criterion Tris-HCl and Criterion XT Bis-Tris Gels, bulletin 5763
- Molecular Weight Estimation and Quantitation of Protein Samples Using Precision Plus Protein WesternC Standards, the Immun-Star WesternC Chemiluminescent Detection Kit, and the Molecular Imager ChemiDoc XRS Imaging System, bulletin 5576

Links

- Image Lab Software
- Imaging Systems
- Gel Doc XR+ System
- Gel Doc EZ System
- ChemiDoc MP System
- ChemiDoc XRS+ System
- Quantity One 1-D Analysis Software
- PDQuest 2-D Analysis Software

Imaging Software

A robust software package is required for image acquisition to analyze data and draw conclusions from PAGE applications. Sophisticated gel analysis software provides a variety of tools that enhance the user’s ability to evaluate the acquired data. The software adjusts contrast and brightness, magnifies, rotates, resizes, and annotates gel images, which can then be printed using standard and thermal printers. All data in the images can be quickly and accurately quantified. The software can measure total and average quantities and determine relative and actual amounts of protein. Gel imaging software is also capable of determining the presence/absence and up/down regulation of proteins, their molecular weight, pI, and other values. For more information on imagers and gel evaluation software, visit www.bio-rad.com.

Bio-Rad offers three different software packages for gel imaging and analysis:

- Image Lab™ software — image acquisition and analysis software that runs the ChemiDoc MP, Gel Doc XR+, Gel Doc EZ, and ChemiDoc XRS+ imaging systems. The software allows automatic configuration of these imaging systems with appropriate filters and illumination sources. It also allows manual or automated analysis of PAGE gels and western blots
- Quantity One® 1-D analysis software — acquires, quantitates, and analyzes a variety of data, including radioactive, chemiluminescent, fluorescent, and color-stained samples acquired from densitometers, storage phosphor imagers, fluorescence imagers, and gel documentation systems. The software allows automatic configuration of these imaging systems with appropriate filters, lasers, LEDs, and other illumination sources. It also allows manual or automated analysis of PAGE gels and western blots
- PDQuest™ 2-D analysis software — used for 2-D gel electrophoretic analysis

Analysis

Beyond protein band patterns, PAGE can yield information about a protein’s size (molecular weight) and yield (quantity). Image analysis software greatly enhances and facilitates these measurements.

Molecular Weight (Size) Estimation

SDS-PAGE is a reliable method for estimating the molecular weight (MW) of an unknown protein, since the migration rate of a protein coated with SDS is inversely proportional to the logarithm of its MW. The key to accurate MW determination is selecting separation conditions that produce a linear relationship between log MW and migration within the likely MW range of the unknown protein. A protocol for MW estimation is provided in Part II of this guide.

To ensure accurate MW determination:

- Separate the protein sample on the same gel with a set of MW standards (see Chapter 3 for information regarding selection of protein standards)
- For statistical significance, generate multiple data points (>3 lanes per sample)
- Use a sample buffer containing reducing agents (DTT or βME) to break disulfide bonds and minimize the effect of secondary structure on migration
- Include SDS in the sample buffer. SDS denatures secondary, tertiary, and quaternary structures by binding to hydrophobic protein regions. SDS also confers a net negative charge on the proteins, which also results in a constant charge-to-mass ratio

After separation, determine the relative migration distance (R_f) of the protein standards and of the unknown protein. R_f is defined as the mobility of a protein divided by the mobility of the ion front. Because the ion front can be difficult to locate, mobilities are normalized to the tracking dye that migrates only slightly behind the ion front:

$R_f = (\text{distance to band})/(\text{distance to dye front})$

Using the values obtained for the protein standards, plot a graph of log MW vs. R_f (Figure 6.3). The plot should be linear for most proteins, provided that they are fully denatured and that the gel percentage is appropriate for the MW range of the sample. The standard curve is sigmoid at extreme MW values because at high MW, the sieving affect of the matrix is so large that molecules are unable to penetrate the gel. At low MW, the sieving effect is negligible and proteins migrate almost freely. To determine the MW of the unknown protein band, interpolate the value from this graph.

The accuracy of MW estimation by SDS-PAGE is in the range of 5–10%. Glyco- and lipoproteins are usually not fully coated with SDS and will not behave as expected in SDS-PAGE, leading to false estimations. For more details about molecular weight estimation using SDS-PAGE, refer to bulletin 3133.

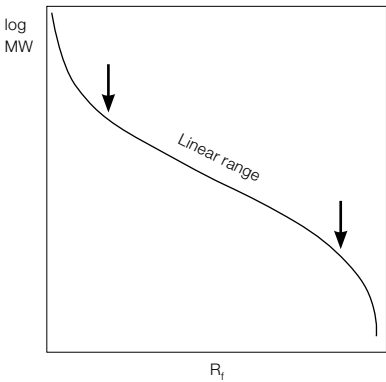


Fig. 6.3. Typical characteristics of a log MW vs. R_f curve for protein standards.

Quantitation

Of all the methods available for protein quantitation (including UV spectroscopy at 280 nm, colorimetric dye-based assays, and electrophoresis in combination with image acquisition analysis), only protein quantitation by electrophoresis enables evaluation of purity, yield, or percent recovery of individual proteins in complex sample mixtures.

Two types of quantitation are possible: relative quantitation (quantitation of one protein species relative to the quantity of another) and absolute quantitation (quantitation of a protein by using a calibration curve generated by a range of known concentrations of that protein). Because proteins interact differently with protein stains, the staining intensity of different proteins at identical protein loads can be very different. Thus, only relative quantitative values can be determined in most cases. Absolute protein measurements can only be made if the protein under investigation is available in pure form and is used as the calibrant.

For protein quantitation using PAGE to be of value:

- Employ sample preparation procedures that avoid nonspecific protein loss due to insolubility, precipitation, and absorption to surfaces
- Ensure all proteins enter the electrophoretic separation medium
- Optimize the quality of the electrophoretic separation. For example, wavy, distorted protein bands and comigration of bands lead to questionable results
- When possible, separate a dilution series of pure proteins in parallel. This enables the creation of a calibration curve (as for molecular weight determination with SDS-PAGE, above)
- Analyze all samples (including samples for calibration) at least in duplicate
- Use a stain that offers sufficient sensitivity and a high dynamic range. Fluorescent stains like Flamingo and Oriole fluorescent gel stains are recommended over Coomassie and silver staining techniques

Links

- Fluorescent Protein Stains
- Flamingo Fluorescent Gel Stain
- Oriole Fluorescent Gel Stain
- Coomassie Stains
- Silver Stains



CHAPTER 7

Downstream Applications

Following electrophoresis, the entire gel might be blotted (proteins transferred to a membrane) or dried, or individual proteins might be excised or eluted from the gel for analysis.

Western Blotting (Immunoblotting)

When specific antibodies are available, transferring the proteins to a membrane (blotting) followed by immunological staining is an attractive complement to general protein stains and provides additional information. A typical immunoblotting experiment consists of five steps (Figure 7.1). Following PAGE:

1. Proteins are transferred from the gel to a membrane where they become immobilized as a replica of the gel's band pattern (blotting).
2. Unoccupied protein-binding sites on the membrane are saturated to prevent nonspecific binding of antibodies (blocking).
3. The blot is probed for the proteins of interest with specific primary antibodies.
4. Secondary antibodies, specific for the primary antibody type and conjugated to detectable reporter groups such as enzymes or radioactive isotopes, are used to label the primary antibodies.
5. Labeled protein bands are visualized by the bound reporter groups acting on an added substrate or by radioactive decay.

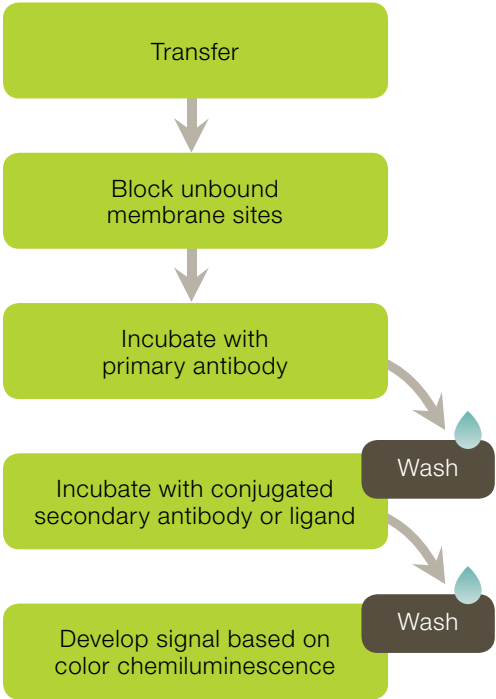


Fig. 7.1. Western blotting workflow.

Bio-Rad offers a complete range of products for blotting, including blotting cells for protein transfers, blotting membranes, filter paper, premixed blotting buffers, reagents, protein standards, and detection kits. Please refer to the Protein Blotting Guide (Bio-Rad bulletin 5294) for more information.

Gel Drying

Before the advent of modern gel imaging equipment, gels were dried in a format that not only preserved them, but made them more amenable to densitometry or autoradiography. The Model 583 and GelAir™ gel dryer systems dry gels between two sheets of cellophane.

Electroelution

Electroelution, as its name implies, is a technique that applies the principles of electrophoresis to enable recovery (elution) of molecules such as proteins from gels and gel slices. It can be used with either slices from a gel containing the protein of interest or with entire preparative gels. Electroelution uses an electrical field and the charged nature of proteins to move them from the gel and into a buffer solution. Once eluted, proteins can be assayed for activity, applied to subsequent purification steps, or subjected to mass spectrometry or a variety of other applications.

The Model 422 electro-eluter (Figure 7.2) combines with the tank and lid of the Mini Trans-Blot® cell (or older Mini-PROTEAN® II or Mini-PROTEAN 3 cells) to elute macromolecules from single or multiple gel slices. The electro-eluter has six vertical glass tubes connecting the upper and lower buffer chambers. A frit at the bottom of each tube retains the gel slice but permits macromolecules to migrate through when current is applied. When the macromolecules have passed through the frit, they are collected in the membrane cap for further analysis or testing. Depending on the buffer system, the Model 422 electro-eluter can be used for elution or dialysis of up to six samples.



Fig. 7.2. Model 422 electro-eluter

The whole gel eluter and mini whole gel eluter (Figure 7.3, Table 7.1) simultaneously elute and collect protein fractions from preparative slab gels into liquid fractions (Figure 7.4). Applications include screening of crude mixtures of proteins to identify immunologically relevant antigens and purification of multiple bands of nucleic acids or proteins.



Fig. 7.3. Whole gel eluter and mini whole gel eluter.

Table 7.1. Whole gel eluter specifications.

| | Whole Gel Eluter | Mini Whole Gel Eluter |
|-----------------|------------------|-----------------------|
| Gel area | 14 x 16 cm | 6.5 x 5.5 cm |
| Fraction volume | 30 x 3 ml | 14 x 0.5 ml |
| Sample load | mg | µg |

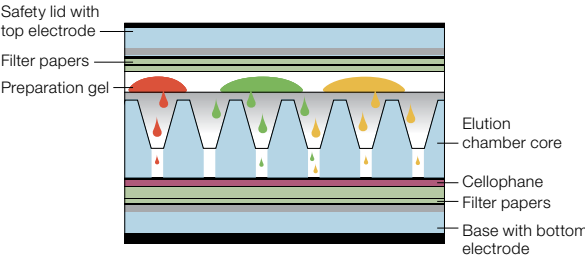


Fig. 7.4. Whole gel eluter and mini whole gel eluter elute protein bands into discrete liquid fractions. Proteins are electrophoretically eluted from the gel into precisely spaced channels of the elution chamber core. Liquid fractions are then harvested into a harvesting box.

Spot Excision (Cutting)

Bands containing proteins of interest can be excised from gels either by hand (for example, using a razor blade) or with the help of automated spot cutting systems, such as those commonly used in 2-D gel-based proteomics workflows. The proteins in the bands can then be either eluted from the gel piece (for example, by electroelution) or subjected to downstream processing (for example, tryptic digestion) while still in the gel.

Automated spot cutting instruments (spot cutters) are used to excise protein spots of interest from gels and to transfer them to microplates or other vessels for further analysis. Bio-Rad's EXQuest™ spot cutter (Figure 7.5) locates and excises protein bands or spots from 1-D and 2-D gels or blots and loads them into 96- and 384-well microplates or 96-tube racks for downstream processing and analysis. The spot cutter can be fully integrated with the PDQuest™ 2-D analysis software or Quantity One® 1-D analysis software, and it works with the imaging system to visualize gels and blots that are either visibly or fluorescently stained. In 2-D PAGE applications, PDQuest software tracks the protein bands or spots through spot cutting and mass spectrometric protein analysis.

The EXQuest spot cutter allows the use of the following common proteome separation and staining methods:

- Freestanding, plastic- or glass-backed 2-D and 1-D SDS-PAGE gels
- PVDF and nitrocellulose membrane blots
- Gels or membranes stained for proteins with visible stains (such as silver and Coomassie blue stains) or fluorescent stains (such as Flamingo™, Oriole™, and SYPRO Ruby protein stain)



Fig. 7.5. EXQuest spot cutter.



TABLE OF CONTENTS

Part II Methods

Protocols

Sample Preparation

General Tips for Sample Preparation

Keep the sample preparation workflow simple (increasing the number of sample handling steps may increase variability).

Lysis (Cell Disruption)

- Suspend ~1 mg (wet weight) pelleted cells in ~10 µl SDS-PAGE sample buffer for a protein concentration of 3–5 µg/µl. If disrupted in liquid nitrogen, tissue samples like liver biopsies and plant leaves contain 10–20% and 1–2% protein, respectively
- To diminish endogenous enzymatic activity:
 - Disrupt the sample or place freshly disrupted samples in solutions containing strong denaturing agents such as 7–9 M urea, 2 M thiourea, or 2% SDS. In this environment, enzymatic activity is often negligible
 - Perform cell disruption at low temperatures to diminish enzymatic activity
 - Lyse samples at pH >9 using either sodium carbonate or Tris as a buffering agent in the lysis solution (proteases are often least active at basic pH)
 - Add a chemical protease inhibitor to the lysis buffer. Examples include phenylmethylsulfonyl fluoride (PMSF), aminoethyl-benzene sulfonyl fluoride (AEBSF), tosyl lysine chloromethyl ketone (TLCK), tosylphenylchloromethylketone (TPCK), ethylenediaminetetraacetic acid (EDTA), benzamidine, and peptide protease inhibitors (for example, leupeptin, pepstatin, aprotinin, and bestatin). For best results, use a combination of inhibitors in a protease inhibitor cocktail
 - If protein phosphorylation is to be studied, include phosphatase inhibitors such as fluoride and vanadate
- When working with a new sample, use at least two different cell disruption protocols and compare the protein yield (by protein assay) and qualitative protein content (by SDS-PAGE)
- Optimize the power settings of mechanical rupture systems and incubation times for all lysis approaches. Because mechanical cell lysis usually generates heat, employ cooling where required to avoid overheating of the sample
- Following cell disruption, check the efficacy of cell wall disruption by light microscopy and centrifuge all extracts extensively (20,000 x g for 15 min at 15°C) to remove any insoluble material; solid particles may block the pores of the electrophoresis gel

Protein Solubilization

- Solubilize proteins in a buffer that is compatible with the corresponding electrophoresis technique
- Dissolve pelleted protein samples in 1x sample buffer
- Dilute dissolved protein samples with sample buffer stock solutions to a final sample buffer concentration of 1x
- Perform a protein quantitation assay to determine the amount of total protein in each sample. Use a protein assay that is tolerant to chemicals in your samples. For samples in Laemmli sample buffer, for example, use the *DC™* or *RC DC™* protein assays, which can tolerate up to 10% detergent. Omit the protein assay if sample amount is limited.
- Dilute or concentrate samples as needed to yield a final protein concentration of >0.5 mg/ml
- Use protein extracts immediately or aliquot them into appropriately sized batches and store them at –80°C to avoid freeze-thaw cycles
- For long-term sample storage, store aliquots at –80°C; avoid repeated thawing and freezing of protein samples
- Highly viscous samples likely have a very high DNA or carbohydrate content. Fragment DNA with ultrasonic waves during protein solubilization or by adding endonucleases like benzonase. Use protein precipitation with TCA/acetone (for example, with the ReadyPrep™ 2-D cleanup kit) to diminish carbohydrate content
- When a sample preparation protocol calls for a dilution, the two parts are stated like a ratio, but what is needed is a fraction. For example, “Dilute 1:2,” means to take 1 part of one reagent and mix with 1 part of another, essentially diluting the part by half. “Dilute 1:4,” means to take 1 part and mix with 3 parts, making a total of 4 parts, diluting the part by a quarter

Preparation for PAGE

- Prepare SDS-PAGE sample buffer without reducing agent, then aliquot and store at room temperature
- Prepare fresh reducing agent, and add it to SDS-PAGE sample buffer immediately before use
- Dissolve dry protein samples directly in 1x sample buffer; prepare other protein samples such that the final sample buffer concentration is 1x
- Incubate samples in sample buffer at 95°C for 5 min (or at 70°C for 10 min) after addition of sample buffer for more complete disruption of molecular interactions
- When preparing SDS-PAGE sample buffer, use either 5% (~100 mM) 2-mercaptoethanol (βME) or 5–10 mM dithiothreitol (DTT)
- The final protein concentration in the sample solution for 1-D electrophoresis should not be <0.5 mg/ml

Protocols

Sample Preparation

Human Cells

Use the MicroRotor™ cell lysis kit (mammalian) or the protocol detailed here, which uses sonication and radioimmunoprecipitation assay (RIPA) buffer, for cell lysis and protein extraction.

Suspension Cultured Cells

- 1
- Pellet the cells by centrifugation at 2,000 × g for 5 min at 4°C.
- 2
- Discard the supernatant and wash pelleted cells in cold PBS. Repeat steps 1 and 2 twice.
- 3
- Add RIPA buffer to the pelleted cells and suspend the pellet with a pipet.

Monolayer Cultured Cells

- 1
- Carefully remove (decant) culture medium from cells. Wash cells twice in cold PBS.
- 2
- Add RIPA buffer to the cells and keep on ice for 5 min. Swirl the plate occasionally to spread the buffer around the plate.
- 3
- Use a cell scraper to collect the lysate and transfer to a microcentrifuge tube.

- 4
- Place the cell suspension on ice, incubate 5 min, and sonicate at appropriate intervals. Check lysis efficacy by light microscopy.
- 5
- Centrifuge cell debris at ~14,000 × g for 15 min at 4°C and transfer supernatant to a new vial.
- 6
- Perform a protein assay of the supernatant. A protein concentration of 3–5 µg/µl is best for PAGE.
- 7
- Add 2x SDS-PAGE sample buffer to the protein solution to yield a 1x sample buffer concentration.

Reagents

- Phosphate buffered saline (PBS)
- RIPA solubilization buffer (use 1 ml RIPA buffer with 3 × 10⁷ cells; store and use RIPA buffer at 4°C
- SDS-PAGE sample buffer (2x)

Equipment

- Centifuge
- Sonicator

Links

- [DC Protein Assay](#)
- [RC DC Protein Assay](#)
- [SDS-PAGE Sample Buffer](#)
- [MicroRotor Cell Lysis Kit \(Mammal\)](#)
- [ReadyPrep 2-D Cleanup Kit](#)

Protocols

Sample Preparation

Tips

- Use liquid nitrogen and a mortar and pestle to grind the samples while they are still frozen. Mill any larger pieces beforehand (for example, wrap the frozen tissue sample in aluminum foil and crush with a hammer)
- With plant leaves, precipitate proteins with 20% TCA in prechilled acetone (–20°C). To remove the plant phenols, rinse the pellet at least twice with cold acetone (–20°C) and air-dry samples in a vacuum

Reagents

- Mammalian Tissue**
- Lysis buffer
 - SDS-PAGE sample buffer (2x)
- Plant Leaves**
- Protein precipitation solution
 - Wash solution
 - SDS-PAGE sample buffer (1x)

Equipment

- Mortar and pestle

Links

- [MicroRotofor Cell Lysis Kit \(Mammal\)](#)
- [SDS-PAGE Sample Buffer](#)
- [MicroRotofor Cell Lysis Kit \(Plant\)](#)

Mammalian Tissue

Use the MicroRotofor cell lysis kit (mammal). Alternatively, use the protocol here, which involves freezing mammalian tissue samples (for example, biopsy samples) in liquid nitrogen at –196°C.

1

Chill a mortar with liquid nitrogen, then grind small tissue pieces in the presence of liquid nitrogen to a fine powder.

2

Immediately after grinding, transfer 60 mg tissue powder to a microcentrifuge tube containing 1.0 ml lysis buffer.

3

Optional: sonicate the sample on ice 5 times, for 2 sec every time. Pause between sonication steps to avoid overheating.

4

Incubate the sample at room temperature for 30 min. Vortex from time to time.

5

Centrifuge at 35,000 x g for 30 min at room temperature.

6

Perform a protein assay to determine the protein concentration of the supernatant, which should be 5–10 µg/µl.

7

Dilute supernatant with 2x SDS-PAGE sample buffer (to a final 1x concentration), and incubate for 20 min at room temperature. Do not heat the sample.

Plant Leaves

To minimize the deleterious effects of endogenous plant compounds, use the MicroRotofor cell lysis kit (plant) or the protocol here, which involves grinding the tissue in a mortar and pestle with liquid nitrogen.

1

Cool protein precipitation and wash solutions to –20°C. Chill a mortar with liquid nitrogen.

2

Place 3–4 leaves in the mortar, add liquid nitrogen, and grind the leaves in the liquid nitrogen to a fine powder.

3

Transfer leaf powder into 20 ml of protein precipitation solution and incubate for 1 hr at –20°C. Stir occasionally.

4

Centrifuge the solution at –20°C for 15 min at 35,000 x g. Discard supernatant, add wash solution and suspend the pellet. Incubate for 15 min at –20°C, stirring occasionally. Repeat until wash solution turns light green.

5

Centrifuge the solution at –20°C for 15 min at 35,000 x g and discard supernatant. Resuspend pellet in 2 ml wash solution.

6

Transfer suspension into a shallow ceramic shell and cover with perforated parafilm. Put shell into a dessicator and apply a vacuum until the pellet (acetone powder) is dry.

7

Mix 3 mg of sample powder with 1 ml 1x SDS-PAGE sample buffer and incubate for 30 min at room temperature. Vortex from time to time.

8

Centrifuge the solution at –20°C for 15 min at >16,000 x g. Collect and heat the supernatant for 3 min at 95°C.

9

Cool solution to room temperature and perform the protein assay.

Protocols

Sample Preparation

Microbial Cultures

Use the MicroRotofor cell lysis kit (bacteria) or the MicroRotofor cell lysis kit (yeast). Alternatively, use the protocol detailed here, which relies on cell lysis with ultrasonic waves in combination with a solubilization in SDS under elevated temperature. This ensures deactivation and denaturation of proteases.

1

Centrifuge cells (~5 x 10⁷) for 3 min at 5,000 x g and resuspend the pellet in an equal volume of 37°C PBS and centrifuge again. Repeat two more times to remove all interfering material (extracellular proteases and growth media).

2

Add 200 µl of hot (95°C) SDS sample solubilization buffer to the pellet and vortex thoroughly.

3

Sonicate the sample solution 10 times for 1 sec each at ~60 W and ~20 kHz. Incubate at 95°C for 5 min.

4

Cool the sample to 20°C and dilute with ~250 µl 2x SDS-PAGE sample buffer. Incubate for another 20 min at room temperature.

5

Centrifuge the sample solution at 20°C for 30 min at 14,000 x g and harvest the supernatant.

6

Perform the protein assay. The protein concentration should be ~5 µg/µl.

Protein Fractions from Chromatography

When checking fraction purity or the enrichment of a particular protein after a chromatographic separation, you can observe the presence of high concentrations of salt, detergent, denaturants, and organic solvents. For example, in ion exchange chromatography, proteins are eluted by a salt gradient. But the salt concentration of the corresponding fractions can be as high as 0.5 M, a concentration that interferes with SDS-PAGE.

Remove salt and other contaminants by one of the following approaches:

- Buffer exchange — use Bio-Spin® or Micro Bio-Spin™ columns, which are filled with size exclusion media equilibrated in Tris buffer. These columns accommodate a sample volume 50–100 µl and remove compounds <6 kD within 10 min. Mix the purified sample with 2x SDS-PAGE sample buffer
- Precipitation — use the ReadyPrep™ 2-D cleanup kit (based on an acetone/TCA precipitation) for simultaneous removal of interfering substances and concentration of dilute samples (<50 ng/ml)

Tips

- Reproducible lysis and protein solubilization of bacteria and yeast is challenging because the cells may release proteases and other enzymes into the growth medium. Wash the cultures thoroughly with isotonic buffers and take precautions to inactivate the proteolytic activity after cell lysis. Extensive disruption of microbial cells is required, usually with the help of a French press, bead impact instruments, or sonicator

Reagents

- SDS sample solubilization buffer
- SDS-PAGE sample buffer (2x)
- Phosphate buffered saline (PBS)

Equipment

- Centifuge
- Sonicator

Links

- [MicroRotofor Cell Lysis Kit \(Bacteria\)](#)
- [MicroRotofor Cell Lysis Kit \(Yeast\)](#)
- [SDS-PAGE Sample Buffer \(2x\)](#)
- [Bio-Spin and Micro Bio-Spin Columns](#)
- [ReadyPrep 2-D Cleanup Kit](#)

Protocols

Sample Quantitation (RC DC Protein Assay)

The RC DC protein assay is based on a modification of the Lowry protocol (Lowry et al. 1951), and is both reducing agent compatible (RC) and detergent compatible (DC). Protein quantitation can be performed in complex mixtures including Laemmli buffer. It involves addition of detection reagents to a protein solution and subsequent measurement of absorbance at 750 nm with a spectrophotometer. Comparison to a standard curve provides a relative measurement of protein concentration.

Tips

- Prepare a standard curve each time the assay is performed
- For best results, prepare the standards in the same buffer as the sample

Links

- Criterion Cell
- Mini-PROTEAN Cell
- PROTEAN II xi Cell
- RC DC Protein Assay
- SmartSpec™ Plus Spectrophotometer

Standard Assay Protocol (5 ml)

- 1

Add 20 µl of DC Reagent S to each 1 ml of DC Reagent A needed. This solution is referred to as Reagent A'. Each standard or sample assayed requires 510 µl Reagent A'.
- 2

Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
- 3

Pipet 100 µl of protein standard or sample into clean tubes. Add 500 µl of RC Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
- 4

Add 500 µl of RC Reagent II to each tube and vortex. Centrifuge the tubes at 15,000 x g for 3–5 min.
- 5

Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
- 6

Add 510 µl of Reagent A' to each tube and vortex. Incubate tubes at room temperature for 5 min, or until the precipitate is dissolved. Vortex.
- 7

Add 4 ml of DC Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.
- 8

Read absorbance of each sample at 750 nm. The absorbances are stable for at least 1 hr.
- 9

Plot absorbance measurements as a function of concentration for the standards.
- 10

Interpolate the concentration of the protein samples from the plot and sample absorbance measurements.

Microfuge Tube Assay Protocol (1.5 ml)

- 1

Add 5 µl of DC Reagent S to each 5 µl of DC Reagent A needed. This solution is referred to as Reagent A'. Each standard or sample assayed requires 127 µl Reagent A'.
- 2

Prepare 3–5 dilutions of a protein standard (0.2–1.5 mg/ml protein).
- 3

Pipet 25 µl of protein standard or sample into clean microcentrifuge tubes. Add 125 µl of RC Reagent I into each tube and vortex. Incubate the tubes for 1 min at room temperature.
- 4

Add 125 µl of RC Reagent II into each tube and vortex. Centrifuge the tubes at 15,000 x g for 3–5 min.
- 5

Discard the supernatant by inverting the tubes on clean, absorbent tissue paper. Allow the liquid to drain completely from the tubes.
- 6

Add 127 µl of Reagent A' to each tube and vortex. Incubate tubes at room temperature for 5 min, or until the precipitate is dissolved. Vortex.
- 7

Add 1 ml of DC Reagent B to each tube and vortex immediately. Incubate at room temperature for 15 min.

Protocols

Handcasting Polyacrylamide Gels

Single-Percentage Gels

- 1

Prepare the resolving and stacking gel solutions without APS or TEMED. (Tables 1 and 2; consult the instruction manual for the system you are using for more details.)

Table 1. Volume of resolving gel solution required to fill a gel cassette. Volumes listed are required to completely fill a gel cassette. Amounts may be adjusted depending on the application (with or without comb, with or without stacking gel, etc.).

| Spacer (Gel Thickness) | Mini-PROTEAN®* Cell | Criterion™ Cell | PROTEAN® II xi Cell | |
|---------------------------|------------------------|--------------------|---------------------|---------|
| | | | 16 cm | 20 cm |
| 0.5 mm | — | — | 12.8 ml | 16.0 ml |
| 0.75 mm | 4.2 ml | — | 19.2 ml | 24.0 ml |
| 1.0 mm | 5.6 ml | 15.0 ml | 25.6 ml | 32.0 ml |
| 1.5 mm | 8.4 ml | — | 38.4 ml | 48.0 ml |
| 3.0 mm | — | — | 76.8 ml | 96.0 ml |

* 10 ml of monomer solution is sufficient for two stacking gels of any thickness.

Table 2. Recipes for stacking and resolving gels. Adjust amounts as needed for the format you are using (see Table 1).

| | Stacking Gel | Resolving Gel | | | |
|-----------------------|--------------|---------------|---------|---------------------|--|
| | 4% | 7.5% | 12% | X% | |
| 30% Acrylamide/bis | 1.98 ml | 3.75 ml | 6.0 ml | 0.33 x X ml | |
| 0.5M Tris-HCl, pH 6.8 | 3.78 ml | — | — | — | |
| 1.5M Tris-HCl, pH 8.8 | — | 3.75 ml | 3.75 ml | 3.75 ml | |
| 10% SDS | 150 µl | 150 µl | 150 µl | 150 µl | |
| diH ₂ O | 9 ml | 7.28 ml | 5.03 ml | 11.03–(0.33 x X) ml | |
| TEMED | 15 µl | 7.5 µl | 7.5 µl | 7.5 µl | |
| 10% APS | 75 µl | 75 µl | 75 µl | 75 µl | |
| Total volume | 15 ml | 15 ml | 15 ml | 15 ml | |

- 2

Degas the solution under a vacuum for at least 15 min. While solutions are degassing, assemble the glass cassette sandwich.
- 3

Place a comb into the assembled gel sandwich. With a marker, place a mark on the glass plate 1 cm below the teeth of the comb. This will be the level to which the separating gel is poured. Remove the comb.

General Tips for Handcasting

- Acrylamide and bisacrylamide are neurotoxins when in solution. Avoid direct contact with the solutions and clean up spills
- For casting multiple gels, use the Mini-PROTEAN 3 multi-casting chamber (catalog #165-4110), PROTEAN II multi-gel casting chamber (catalog #165-2025), or PROTEAN Plus multi-casting chamber (catalog #165-4160)
- Use only high-quality reagents, especially acrylamide monomers, to avoid polymerization problems
- Proper degassing and filtering of the casting solution is critical for both reproducibility of the polymerization (oxygen removal) and the avoidance of problems related to mass spectrometry (keratin contamination)
- A temperature of 23–25°C is best for degassing and polymerization; equilibrate the stock solutions to room temperature
- APS/TEMED-initiated reactions should proceed for at least 2 hr to ensure maximum reproducibility of pore size
- Make fresh APS solution every day for best performance
- Replace TEMED every three months because it is subject to oxidation, which causes the gradual loss of catalytic activity
- The glass plates must be clean and free of chips. Clean glass plates with ethanol and lint-free cloths before use
- The height of the stacking gel should be at least 2x the height of the sample in the well. This ensures band sharpness, even for diluted protein samples
- Store gels flat in the fridge at 4°C. Do not freeze. Wrap handcast gels tightly in plastic wrap with combs still inserted
- Run handcast gels with discontinuous buffer systems right after gel casting because the buffer discontinuity (pH and ionic strength) gradually disappears during gel storage. SDS-PAGE gels are not stable at pH 8.8 over a longer time period
- For more information about acrylamide polymerization, refer to Acrylamide Polymerization – A Practical Approach, bulletin 1156

Protocols

Handcasting Polyacrylamide Gels

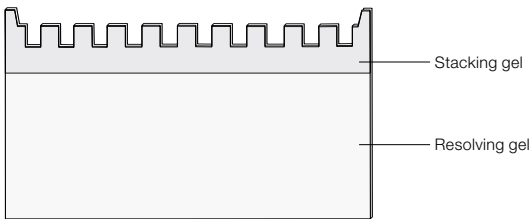
Single-Percentage Gels (contd.)

Pour the Resolving Gel

- 1
- Add the APS and TEMED to the degassed resolving gel solution, and pour the solution to the mark, using a glass pipet and bulb.
- 2
- Using a Pasteur pipet and bulb, immediately overlay the monomer solution with water-saturated *n*-butanol.
- 3
- Allow the gel to polymerize 45–60 min. The gel is polymerized once you see a line form between the stacking and the resolving gel. Pour off the overlay solution and rinse the top of the gel with diH₂O.

Alternative Casting Procedure

It is possible to cast separation and stacking gels one after another, with no intermediate step requiring overlay solution (water-saturated *n*-butanol). Recalculate your gel casting recipes so that the separation gel solution contains 25% (w/v) glycerol. Due to the significant difference in density, the two solutions won't mix when the stacking gel solution is carefully poured on top of the resolving gel solution.



Pour the Stacking Gel

- 1
- Dry the area above the separating gel with filter paper before pouring the stacking gel.
- 2
- Place the comb in the cassette and tilt it so that the teeth are at a slight (~10°) angle. This prevents air from becoming trapped under the comb while the acrylamide solution is being poured.
- 3
- Add the APS and TEMED to the degassed resolving gel solution, and pour the solution down the spacer nearest the upturned side of the comb. Pour until all the teeth are covered by the solution.
- 4
- Realign the comb in the sandwich and add monomer to fill the cassette completely. An overlay solution is not necessary for polymerization when a comb is in place.
- 5
- Allow the gel to polymerize 30–45 min.
- 6
- Remove the comb by pulling it straight up slowly and gently. Rinse the wells completely with diH₂O.

Tips

- When pouring the resolving gel solution, pour the solution down the middle of the outside plate of the gel sandwich or down the side of one of the spacers. Pour smoothly to prevent it from mixing with air
- For the overlay solution, water, *n*-butanol, or *t*-amyl alcohol can also be used. With *n*-butanol or *t*-amyl alcohol, the overlay solution can be applied rapidly because very little mixing will occur. If using water to overlay, use a needle and syringe and a steady, even rate of delivery to prevent mixing
- Do not allow alcohols to remain on the gels for more than 1 hr or dehydration of the top of the gel will occur. It is sometimes convenient to cast the separating portion of the discontinuous gel the afternoon before casting the stacking gel and running the gel. If the stacking gel is to be cast the following day, place approximately 5 ml of 1:4 diluted running gel buffer on top of each separating gel after rinsing with deionized water to prevent dehydration of the separating gel

Links

Polyacrylamide Gel Reagents

Protocols

Handcasting Polyacrylamide Gels

Gradient Gels

This protocol is for preparing 12 mini-format linear gradient gels. It requires the Model 485 gradient former and Mini-PROTEAN 3 multi-casting chamber. For other protocols, refer to the instruction manual for the gradient former you are using.

- 1
- Determine the volume of acrylamide to prepare (≥40 ml is required for the Model 485 gradient former). Prepare the required volume (+5 ml) of acrylamide. Table 3 provides estimated volumes for the casting of 12 mini-format gels. Assemble the stack of Mini-PROTEAN 3 cassettes as described in the Mini-PROTEAN 3 multi-casting chamber instruction manual. Then, flow water through the stopcock and measure the volume required to fill the cassettes. Disassemble the chamber and dry all components.

Table 3. Volume of acrylamide required for 12 mini-format gels. Prepare the amount listed below plus an additional 5 ml.

| Spacer Plates | Volume Required for 12 Gels | Volume to Prepare |
|---------------|-----------------------------|-------------------|
| 0.75 mm | 80 ml | 85–90 ml |
| 1.0 mm | 100 ml | 105–110 ml |
| 1.5 mm | 140 ml | 145–150 ml |

Table 4. Preparation of light and heavy acrylamide solutions.

| Light Solution (4%) | | |
|--|--|-------------|
| 30% Acrylamide stock (30%)(X ml) = (4%)(55 ml) | | X = 7.3 ml |
| 1.5 M Tris-HCl stock buffer, pH 8.8 (1.5 M)(X ml) = (0.375 M)(55 ml) | | X = 13.8 ml |
| Water (55 ml) – (7.3 ml + 13.8 ml) = X | | X = 34 ml |
| 10% APS (500 µl)/(100 ml) = (X µl)/(55 ml) | | X = 275 µl |
| TEMED 10% APS volume; (275 µl)/10 = X | | X = 27.5 µl |

| Heavy Solution (20%) | | |
|--|--|-------------|
| 30% Acrylamide stock (30%)(X ml) = (20%)(55 ml) | | X = 36.7 ml |
| 1.5 M Tris-HCl stock buffer, pH 8.8 (1.5 M)(X ml) = (0.375 M)(55 ml) | | X = 13.8 ml |
| Water (55 ml) – (36.7 ml + 13.8 ml) = X | | X = 4.5 ml |
| APS (500 µl)/(100 ml) = (X µl)/(55 ml) | | X = 275 µl |
| TEMED 10% APS volume; (275 µl)/10 = X | | X = 27.5 µl |

- 2
- Determine the chamber volumes. To create a linear gradient, the volume in each chamber is half the total gel volume required (or 20 ml, whichever is greater). As an example, casting twelve 1.0 mm gels requires 100 ml, so prepare 105 ml (step 1). Divide that volume by two to determine the volume required for each chamber of the gradient former (52.5 ml each for the light and heavy chambers).
- 3
- Determine the heavy and light acrylamide formulations using the chart on the right. (see Table 4) Reassemble the multi-casting chamber.
- 4
- Place the gradient former on a magnetic stir plate and add a magnetic stir bar to the mixing chamber labeled “light.” Attach the luer fitting to the stopcock valve on the inlet port. Run a piece of Tygon tubing (1/8" ID Tygon tubing works well) from the gradient former to the luer fitting on the multi-casting chamber.
- 5
- Combine all reagents except the initiators, and degas the solution for 15 min.
- 6
- Just prior to pouring, add TEMED and APS to both solutions and mix gently. Pour the appropriate monomer solutions into the gradient chambers. (Consult the gradient former instruction manual for complete instructions.) Pour the light solution into the mixing chamber labeled “light,” and the heavy solution in the reservoir chamber labeled “heavy.”
- 7
- Turn on the stirring bar in the mixing chamber, open the tubing clamp of the gradient maker and the stopcock valve of the casting chamber, and pour the gels.

Tips

- If gravity flow isn't fast enough, use a peristaltic pump to pump the entire set of gradients within 10 min. If it is not possible to complete the operation in 10 min from the time initiators are added, then it might be necessary to reduce the amount of initiators (use half the amount of TEMED) to slow polymerization. The gradient should be poured as quickly as possible, without mixing the gradient solution in the casting chamber

Links

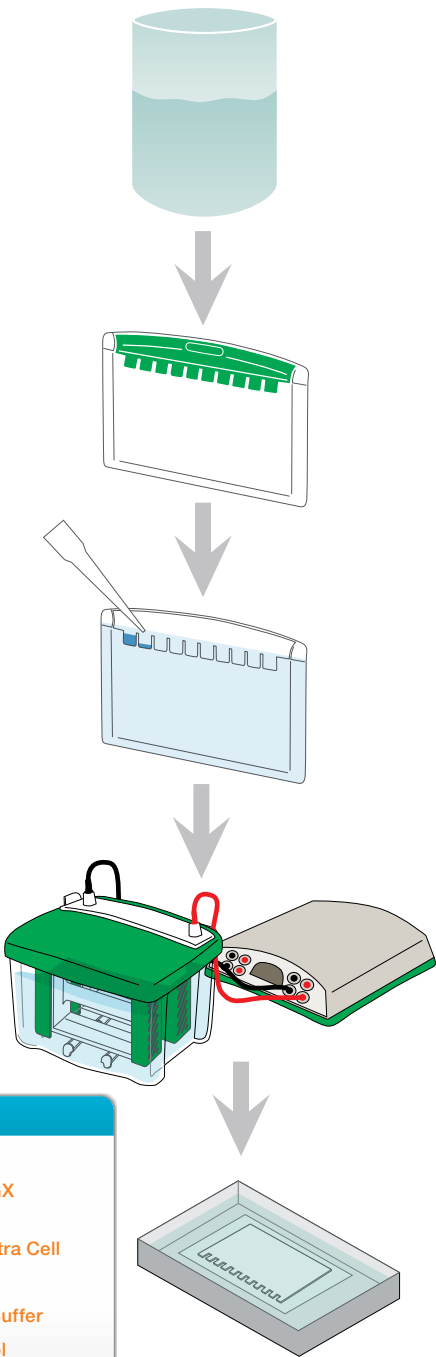
Mini-PROTEAN 3 Multi-Casting Chamber
Model 485 Gradient Former

Protocols

Performing Electrophoresis

General Protocol: SDS-PAGE

The following is a generalized protocol for running a Mini-PROTEAN® TGX™ gel in the Mini-PROTEAN Tetra cell. For detailed instructions, refer to the Mini-PROTEAN precast gels instruction manual and application guide (bulletin #1658100).



- 1
- 2
- 3
- 4
- 5
- 6
- 7
- Prepare buffers:
a. Running buffer (1x): Dilute 100 ml of 10x stock with 900 ml diH₂O.
b. Sample buffer: Use Laemmli sample buffer.
- Prepare gels and assemble the electrophoresis cell:
a. Remove the comb and tape from the gels and assemble the electrophoresis cell.
b. Fill the inner and outer buffer chambers with running buffer. Fill the upper (inner) buffer chamber of each core with 200 ml of 1x running buffer. Fill the lower (outer) buffer chamber to the indicator mark for 2 gels (550 ml) or 4 gels (800 ml) with 1x of running buffer. At runs >200 V, fill the outer buffer chamber to the 4-gel (800 ml) mark.
- Prepare samples as indicated in the table below.
- Heat samples at 90–100°C for 5 min (or at 70°C for 10 min). Load the appropriate volume of your protein sample on the gel.
- Connect the electrophoresis cell to the power supply and perform electrophoresis according to the following conditions:
Run conditions: 200 V
Run time: 31–39 min
Expected current (per gel): Initial 35–50 mA
Final 20–31 mA
- These conditions are for Tris-HCl SDS-PAGE gels. If using Bio-Rad's Mini-PROTEAN TGX gels, the gels can be run at 300 V to decrease the run time.
- After electrophoresis is complete, turn the power supply off and disconnect the electrical leads. Pop open the gel cassettes and remove the gel by floating it off the plate into water.
- Stain and image the gel, using one of the protocols on the following pages as examples.

| Component | Reducing | Nonreducing |
|-----------------------|----------|-------------|
| Sample | 5 µl | 5 µl |
| Laemmli sample buffer | 4.75 µl | 5 µl |
| β-mercaptoethanol | 0.25 µl | — |
| Total volume | 10 µl | 10 µl |

Links

- Mini-PROTEAN TGX Precast Gels
- Mini-PROTEAN Tetra Cell
- Running Buffer
- Laemmli Sample Buffer
- β-Mercaptoethanol
- PowerPac Basic
- Power Supply

Protocols

Performing Electrophoresis

Tips for Electrophoresis

- When preparing running buffers, make the solution as specified in the protocol and do not titrate to a pH. The ion balance is set by the concentration of reagents; adjusting the pH alters this balance and leads to undesirable results
- Do not reuse running buffers
- Use 5–10 V per cm of gel for about 10 min during sample entry (or until the sample has concentrated at the starting point of the separation gel). Then continue with the voltage setting recommended in the instruction manual for the electrophoresis system you are using
- Use the voltage setting recommended in the instruction manual for the electrophoresis system you are using; excessive voltage leads to decreased band resolution, band smearing, and lane distortions
- When running multiple cells, use the same voltage for multiple cells as you would for one cell. Be aware that the current drawn by the power supply will double with two – compared to one – cells. Use a power supply that can accommodate this additive current and set the current limit high enough to permit this additive function
- To maximize reproducibility, maintain the temperature of the electrophoresis buffer at 15°C with the help of a recirculating cooler

Tips for Sample Loading

- The total protein amount loaded per lane depends on the sample complexity and sensitivity of the staining technique. Using 15–20 µg protein per lane for mini- or midi-format gels is a good starting point for complex protein samples when staining with Bio-Safe™ Coomassie stain. Determine the optimum protein load by running a dilution series of the sample
- For best resolution, load a concentrated sample rather than a diluted amount
- Centrifuge the sample solution for 10–15 min at >12,000 x g at 20°C before loading to remove insoluble material that may clog the pores of the acrylamide gel
- To avoid edge effects, add 1x sample buffer to unused wells and never overfill wells
- Load samples either before or after placing the electrophoresis modules into the tank. Both methods produce acceptable results. In both cases, fill both the assembly (inner chamber) and the tank (outer chamber) with buffer
- Add running buffer to the cathode buffer reservoir first and then apply the sample on the stacking gel under the electrode buffer. Sample buffer must contain glycerol to stabilize the sample application zone in the sample well of the gel
- Use pipet tips designed for protein sample loading for best results. For example, Bio-Rad's Prot/Elec™ tips fit easily between vertical slab gel plates of 0.75 mm while maintaining a large bore for fast flow of sample
- Load samples slowly to allow them to settle evenly on the bottom of the well. Be careful not to puncture the bottom of the well with the syringe needle or pipet tip
- If using Bio-Rad's patented sample loading guide, place it between the two gels in the electrode assembly. Sample loading guides are available for 9, 10, 12, and 15-well formats. Use the sample loading guide to locate the sample wells. Insert the Hamilton syringe or pipet tip into the slots of the guide and fill the corresponding wells

Links

- Prot/Elec Tips

TIPS

- Always wear gloves during the staining process. Try to avoid touching the gels with your fingers. Wet gloves with water or buffer before handling the gel to keep the gel from sticking and tearing
- Use clean and dust-free containers for gel staining. If possible, place a lid on the container to avoid contamination of the staining solution
- Gently agitate the container on a horizontal shaker, making sure the gel is completely covered with stain solution all the time
- Use pure chemicals and highly purified diH₂O (conductivity <2 µS)
- Fluorescent dyes like Flamingo and Oriole fluorescent gel stains have a higher dynamic range than Coomassie or silver staining techniques, making them suitable for quantitative protein analysis
- Gels stained with fluorescent dyes can be counterstained with colloidal Coomassie for further reference. In fact, doing so enhances sensitivity of the colloidal Coomassie stain
- For long term-storage, shrink-wrap the stained gels in a 10% glycerol solution (storage at 4°C) or dry them with a GelAir™ drying system within 60 min

Links

- Mini-PROTEAN Gels
- Coomassie Stains
- Bio-Safe Coomassie Stain
- Fluorescent Protein Stains
- Flamingo Fluorescent Gel Stain
- Oriole Fluorescent Gel Stain
- Silver Stains
- GelAir Drying System

Protocols

Total Protein Staining

General protocols are described below for Mini-PROTEAN gels. For more details, refer to the instruction manual for the stain you are using.

Bio-Safe™ Coomassie Stain

- 1
- Wash gels three times for 5 min each in 200 ml diH₂O per gel.
- 2
- Remove all water from staining container and add 50 ml of Bio-Safe Coomassie stain (or enough to completely cover gel). Agitate for 1 hr.
- 3
- Rinse in 200 ml diH₂O for ≥30 min. Stained gels can be stored in water.

Oriole™ Fluorescent Gel Stain

- 1
- If using the 5 L configuration, prepare Oriole stain solution by adding 400 ml of methanol to the 1 L bottle of diluents. Then add 10 ml of Oriole fluorescent gel stain concentrate and mix well by shaking.
- 2
- Place gel in a staining tray with 50 ml of Oriole fluorescent gel stain. Cover the tray, place on a rocker, and agitate gently for ~1.5 hr.
- 3
- Transfer the gel to diH₂O prior to imaging. Destaining is not necessary.

Flamingo™ Fluorescent Gel Stain

- 1
- Place gel in a staining tray with 100 ml of fixing solution (40% ethanol, 10% acetic acid). Cover the tray, place on a rocker, and agitate gently for at least 2 hr.
- 2
- Pour off the fix solution and add 50 ml of 1x stain solution (dilute 1 part Flamingo fluorescent gel stain with 9 parts diH₂O). Cover the tray, place on a rocker or shaker and agitate gently. Stain for at least 3 hr.
- 3
- (Optional) Carefully pour off the stain solution and replace with an equal volume of 0.1% (w/v) Tween 20. Cover the tray, place on a rocker or shaker and agitate gently for 10 min.
- 4
- Rinse gel with diH₂O prior to imaging.

Silver Staining (Bio-Rad Silver Stain)

| Step | Reagent | Volume | Duration | | |
|------|---|------------------|--|----------------|-------------|
| | | | <0.5 mm Gel | 0.5–1.0 mm Gel | >1.0 mm Gel |
| 1 | Fixative 40% methanol/10% acetic acid | 400 ml | 30 min | 30 min | 60 min |
| 2 | Fixative 10% ethanol/5% acetic acid | 400 ml | 15 min | 15 min | 130 min |
| 3 | | 400 ml | 15 min | 15 min | 130 min |
| 4 | Oxidizer diH ₂ O | 200 ml 400 ml | 3 min 2 min | 5 min | 10 min |
| 5 | | 400 ml | 2 min | 5 min | 10 min |
| 6 | | 400 ml | 2 min | 5 min | 10 min |
| 7 | (Repeat washes 5–7 times until all the yellow color is gone from the gel) | 400 ml | 2 min | 5 min | 10 min |
| 8 | Silver reagent | 200 ml | 15 min | 20 min | 30 min |
| 9 | diH ₂ O | 400 ml | — | 1 min | 2 min |
| 10 | Developer | 200 ml | ~30 sec. Develop until solution turns yellow or until brown precipitate appears. | | |
| 11 | | 200 ml | ~5 min | ~5 min | ~5 min |
| 12 | | 200 ml | — | ~5 min | ~5 min |
| 13 | Stop 5% acetic acid | 400 ml | ~5 min | ~5 min | ~5 min |

Links

- Quantity One 1-D Analysis Software
- Precision Plus Protein Unstained Standards
- Criterion Tris-HCl Precast Gels
- Bio-Safe Coomassie Stain

Protocols

Molecular Weight Estimation

Run the standards and samples on an SDS-PAGE gel. Process the gel with the desired stain and then destain to visualize the protein bands. Determine the R_f graphically or using Quantity One® analysis software (or equivalent).

Figures 1 and 2 illustrate the procedure. To determine MW graphically:

- 1
- Using a ruler, measure the migration distance from the top of the resolving gel to each standard band and to the dye front.
- 2
- For each band in the standards, calculate the R_f value using the following equation:

 $R_f = \frac{\text{migration distance of the protein}}{\text{migration distance of the dye front}}$
- 3
- Repeat this step for the unknown bands in the samples.
- 4
- Using a graphing program, plot the log (MW) as a function of R_f.
- 5
- Generate the equation $y = mx + b$, and solve for y to determine the MW of the unknown protein.

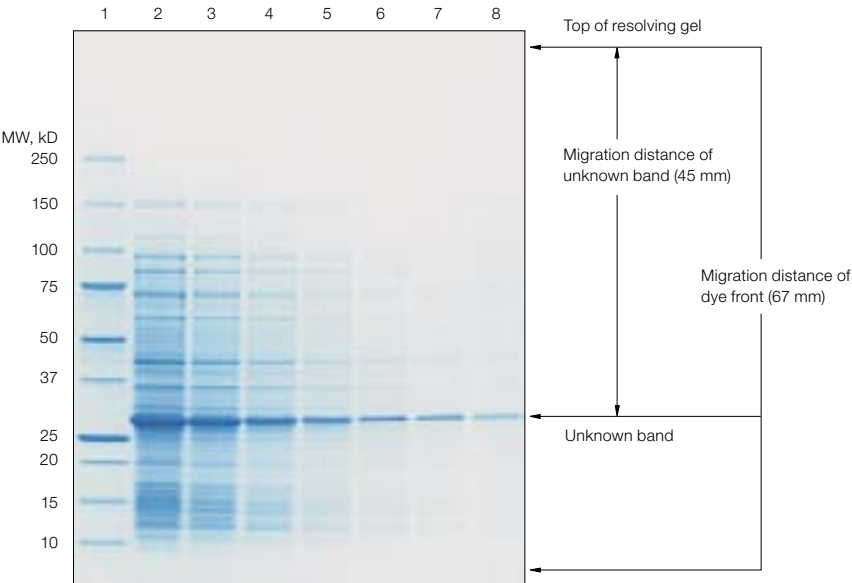


Fig. 1. Example showing MW determination of an unknown protein. Lane 1, 10 µl of Precision Plus Protein™ unstained standards; lanes 2–8, a dilution series of an *E. coli* lysate containing a hypothetical unknown protein (GFP). Proteins were separated by SDS-PAGE in a Criterion™ 4–20% Tris-HCl gel and stained with Bio-Safe Coomassie stain. Gel shown is the actual size.

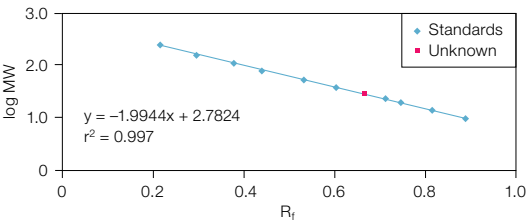


Fig. 2. Determining the MW of an unknown protein by SDS-PAGE. A standard curve of the log (MW) versus R_f was generated using the Precision Plus Protein standards from Figure 1. The strong linear relationship ($r^2 > 0.99$) between the proteins' MW and migration distances demonstrates exceptional reliability in predicting MW.

Buffer Formulations

Sample Preparation Buffers

1 M Tris-HCl, pH 7.6 (100 ml)

| | |
|---|-----------|
| Tris base | 12.11 g |
| Deionized H ₂ O (diH ₂ O) | 80 ml |
| Adjust pH to 7.6 with HCl. | |
| diH ₂ O | to 100 ml |

0.5 M Tris-HCl, pH 6.8 (100 ml)

(catalog #161-0799)

| | |
|----------------------------|-----------|
| Tris base | 6.06 g |
| diH ₂ O | ~60 ml |
| Adjust to pH 6.8 with HCl. | |
| diH ₂ O | to 100 ml |
| Store at 4°C. | |

10% SDS (10 ml)

(catalog #161-0416)

| | |
|--------------------|----------|
| SDS | 1.00 g |
| diH ₂ O | to 10 ml |

1.0% Bromophenol Blue (10 ml)

(catalog #161-0404)

| | |
|--------------------|----------|
| Bromophenol blue | 100 mg |
| diH ₂ O | to 10 ml |

RIPA Solubilization Buffer (100 ml)

25 mM Tris-HCl pH 7.6, 150 mM NaCl, 5 mM EDTA, 1% NP-40 or 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS

| | |
|-----------------------|-----------|
| NaCl | 0.88 g |
| EDTA | 0.15 g |
| NP-40 or Triton X-100 | 1 g |
| Sodium deoxycholate | 1 g |
| SDS | 0.10 g |
| diH ₂ O | 80 ml |
| 1 M Tris-HCl, pH 7.6 | 2.5 ml |
| diH ₂ O | to 100 ml |

Phosphate Buffered Saline (PBS, 1 L)

0.9% (w/v) sodium chloride in 10 mM phosphate buffer, pH 7.4

| | |
|------------------------------------|--------|
| NaCl | 8.00 g |
| KCl | 0.20 g |
| Na ₂ HPO ₄ | 1.44 g |
| KH ₂ PO ₄ | 0.24 g |
| diH ₂ O | 800 ml |
| Adjust pH to 7.4 with HCl or NaOH. | |
| diH ₂ O | to 1 L |

SDS-PAGE Sample Buffer (2x, 8 ml)

(catalog #161-0737, 30 ml)

62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 0.01% bromophenol blue

| | |
|------------------------|---------|
| 0.5 M Tris-HCl, pH 6.8 | 1.0 ml |
| 25% Glycerol | 2.0 ml |
| 1.0% Bromophenol blue | 0.08 ml |
| 10% SDS | 1.6 ml |
| diH ₂ O | 2.92 ml |

Store as 1–2 ml aliquots at –70°C and add β-mercaptoethanol (0.4 ml) or 3% DTT immediately before use.

Protein Precipitation Solution (100 ml)

20% Trichloroacetic acid (TCA), 0.2% DTT in ice-cold acetone (–20°C)

| | |
|-----------------|-----------|
| TCA | 20.00 g |
| DTT | 0.20 g |
| Acetone | 80 ml |
| Dissolve | |
| Acetone | to 100 ml |
| Store at –20°C. | |

Wash Solution (100 ml)

0.2% DTT in ice-cold acetone (–20°C)

| | |
|-----------------|-----------|
| DTT | 0.20 g |
| Acetone | 80 ml |
| Dissolve | |
| Acetone | to 100 ml |
| Store at –20°C. | |

Lysis Buffer (50 ml)

2 M thiourea, 7 M urea, 4% (w/v) CHAPS, 1% (w/v) DTT, 2% (v/v) carrier ampholytes (pH 3–10)

| | |
|----------------------|----------|
| Urea | 21.00 g |
| Thiourea | 7.60 g |
| diH ₂ O | to 45 ml |
| CHAPS | 2.00 g |
| Bio-Lyte® ampholytes | 1.0 ml |
| DTT | 0.50 g |
| diH ₂ O | to 50 ml |

SDS Sample Solubilization Buffer (50 ml)

1% (w/v) SDS, 100 mM Tris-HCl (pH 9.5)

| | |
|-------------------------------------|----------|
| SDS | 0.50 g |
| Tris base | 0.60 g |
| diH ₂ O | 40 ml |
| Titrate to pH 9.5 with diluted HCl. | |
| diH ₂ O | to 50 ml |

Buffer Formulations

Gel Casting Reagents

Acrylamide/Bis (30% T, 2.67% C)

| | |
|--|-----------|
| Acrylamide (29.2 g/100 ml) | 87.60 g |
| N'N'-bis-methylene-acrylamide | 2.40 g |
| diH ₂ O | to 300 ml |
| Filter and store at 4°C in the dark (30 days). | |

Premade alternatives:

Catalog #161-0125, 37.5:1 acrylamide/bis powder
Catalog #161-0158, 30% acrylamide/bis solution

1.5 M Tris-HCl, pH 8.8 (150 ml)

(catalog #161-0798, 1 L)

| | |
|--------------------------------|-----------|
| Tris base (18.15 g/100 ml) | 27.23 g |
| diH ₂ O | 80 ml |
| Adjust to pH 8.8 with 6 N HCl. | |
| diH ₂ O | to 150 ml |
| Store at 4°C. | |

0.5 M Tris-HCl, pH 6.8

(catalog #161-0799, 1 L)

| | |
|---------------------------------|-----------|
| Tris base | 6.00 g |
| diH ₂ O | 60 ml |
| Adjust to pH 6.87 with 6 N HCl. | |
| diH ₂ O | to 100 ml |
| Store at 4°C. | |

10% (w/v) SDS (100 ml)

(catalog #161-0416)

| | |
|-------------------------------|-----------|
| SDS | 10.00 g |
| diH ₂ O | 90 ml |
| Dissolve with gentle stirring | |
| diH ₂ O | to 100 ml |

10% (w/v) APS (fresh daily)

(catalog #161-0700)

| | |
|---------------------|--------|
| Ammonium persulfate | 0.10 g |
| diH ₂ O | 1 ml |

Water-Saturated *n*-Butanol

| | |
|--------------------|-------|
| <i>n</i> -Butanol | 50 ml |
| diH ₂ O | 5 ml |

Combine in a bottle and shake. Use the top phase only.
Store at room temperature.

Sample Buffers

2x SDS-PAGE (Laemmli, 30 ml)

(catalog #161-0737)

62.5 mM Tris-HCl, pH 6.8, 2% SDS, 25% glycerol, 0.01% bromophenol blue, 5% β-mercaptoethanol (added fresh)

| | |
|------------------------|----------|
| 0.5 M Tris-HCl, pH 6.8 | 3.75 ml |
| 50% Glycerol | 15.0 ml |
| 1.0% Bromophenol blue | 0.3 ml |
| 10% SDS | 6.0 ml |
| diH ₂ O | to 30 ml |

Add β-mercaptoethanol (50 μl to 950 μl sample buffer) before use.

2x Native PAGE (30 ml)

(catalog #161-0738)

62.5 mM Tris-HCl, pH 6.8, 40% glycerol, 0.01% bromophenol blue

| | |
|------------------------|----------|
| 0.5 M Tris-HCl, pH 6.8 | 3.75 ml |
| 50% Glycerol | 24.0 ml |
| 1.0% Bromophenol blue | 0.3 ml |
| diH ₂ O | to 30 ml |

2x Tricine (30 ml)

(catalog #161-0739)

200 mM Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Brilliant Blue G-250, 2% β-mercaptoethanol (added fresh)

| | |
|------------------------|----------|
| 0.5 M Tris-HCl, pH 6.8 | 12.0 ml |
| 50% Glycerol | 24.0 ml |
| 10% SDS | 6.0 ml |
| Coomassie Blue G-250 | 12.00 mg |
| diH ₂ O | to 30 ml |

Add β-mercaptoethanol (20 μl to 980 μl sample buffer) before use.

Buffer Formulations

Running Buffers

10x SDS-PAGE (1 L)

(catalog #161-0732)

250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3

| | |
|--------------------|----------|
| Tris base | 30.30 g |
| Glycine | 144.10 g |
| SDS | 10.00 g |
| diH ₂ O | to 1 L |

Do not adjust the pH (~pH 8.3).

10x Native PAGE (1 L)

(catalog #161-0734)

250 mM Tris, 1.92 M glycine, pH 8.3

| | |
|--------------------|----------|
| Tris base | 30.30 g |
| Glycine | 144.10 g |
| diH ₂ O | to 1 L |

Do not adjust the pH (~pH 8.3).

10x Tris-Tricine (1 L)

(catalog #161-0744)

1 M Tris, 1 M Tricine, 1% SDS, pH 8.3

| | |
|--------------------|----------|
| Tris base | 121.10 g |
| Tricine | 179.20 g |
| SDS | 10.00 g |
| diH ₂ O | to 1 L |

Do not adjust the pH (~pH 8.3).

Buffer Components

0.5 M Tris-HCl, pH 6.8 (1 L)

(catalog #161-0799)

| | |
|----------------------------|---------|
| Tris base | 60.60 g |
| diH ₂ O | ~900 ml |
| Adjust to pH 6.8 with HCl. | |
| diH ₂ O | to 1 L |
| Store at 4°C. | |

10% SDS (250 ml)

(catalog #161-0416)

| | |
|--------------------|-----------|
| SDS | 25.00 g |
| diH ₂ O | to 250 ml |

1.0% Bromophenol Blue (10 ml)

(catalog #161-0404)

| | |
|--------------------|----------|
| Bromophenol blue | 100 mg |
| diH ₂ O | to 10 ml |



Part III

Troubleshooting

Electrophoresis is a straightforward technique. However, problems may occasionally arise during the various steps in the electrophoresis workflow. This section highlights potential problems and their causes, and provides potential solutions.

Sample Preparation

| Problem | Cause | Solution |
|------------------------------------|----------------------------------|--|
| Laemmli sample buffer turns yellow | Sample buffer is too acidic | Add Tris base until buffer turns blue again |
| Sample very viscous | High DNA or carbohydrate content | <ul style="list-style-type: none">Fragment DNA with ultrasonic waves during cell lysis and protein solubilizationAdd endonucleases (for example benzonase)Precipitate protein with TCA/acetone (ReadyPrep™ 2-D cleanup kit) to diminish carbohydrate content |

Gel Casting and Sample Loading

| Problem | Cause | Solution |
|---|--|--|
| Leaking during handcasting | Chipped glass plates | Ensure plates are free of flaws |
| | Spacer plate and short plate not level | Ensure plates are aligned correctly |
| | Casting stand gasket dirty, flawed, or worn out | <ul style="list-style-type: none">Wash gasket if it is dirtyReplace flawed or worn out casting stand gaskets |
| Poor well formation | Incorrect catalyst used | <ul style="list-style-type: none">Prepare fresh catalyst solutionIncrease catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED |
| | Monomer solution not degassed (oxygen inhibits polymerization) | Degas monomer solution immediately prior to casting stacking gel |
| Webbing; excess acrylamide behind the comb | Incorrect catalyst concentration | <ul style="list-style-type: none">Prepare fresh catalyst solutionIncrease catalyst concentration of stacking gel to 0.06% APS and 0.12% TEMED |
| Pressure cams on casting frame are difficult to close or make noise when closed | Powder residue has built up at pivot point of pressure cams | Rinse or wipe off powder residue before each use |
| Gel does not polymerize | Too little or too much APS or TEMED | Use 0.05% APS and 0.05% TEMED |
| | Failure to degas | Degas monomer solutions 10–15 min |
| | Temperature too low | Cast at room temperature, warming glass plates if necessary |
| | Poor quality acrylamide or bis | Use electrophoresis-grade reagents |
| | Old APS | Prepare fresh APS |

| Problem | Cause | Solution |
|---------------------------|---|---|
| Swirls in gel | Excess catalysts; polymerization time <10 min | Reduce APS and TEMED by 25% each |
| | Gel inhibition; polymerization time >2 hr | Increase APS and TEMED by 50%; degas |
| Gel feels soft | Low %T | Use different %T |
| | Poor quality acrylamide or bis | Use electrophoresis-grade reagents |
| | Too little cross-linker | Use correct %C |
| Gel turns white | Bis concentration too high | Check solutions or weights |
| Gel brittle | Cross-linker too high | Use correct %C |
| Sample floats out of well | Sample not dense enough | Include 10% glycerol in sample to make it denser than surrounding buffer |
| | Pipetting, loading error | Pipet sample into well slowly. Do not squirt sample quickly into well, as it may bounce off bottom or sides and flow into next well. Do not remove pipet tip from well before last of sample has left tip |

Electrophoresis

| Problem | Cause | Solution |
|---|---|---|
| Current zero or less than expected, and samples do not migrate into gel | Tape at the bottom of precast gel cassette not removed | Remove tape |
| | Insufficient buffer in inner buffer chamber | Fill buffer chamber with running buffer |
| | Insufficient buffer in outer buffer chamber | Fill inner and outer buffer chambers to ensure wells are completely covered |
| | Electrical disconnection | Check electrodes and connections |
| Gels run faster than expected | Running buffer too concentrated and gel temperature too high; incorrect running buffer concentration or type used | Check buffer composition and type |
| | Running or reservoir buffer too dilute | Check buffer protocol and concentrate if necessary |
| | Voltage too high | Decrease voltage by 25–50% |
| Gels run slower than expected | Incorrect running buffer composition or type | Check buffer composition and type |
| | Excessive salt in sample | Desalt sample |
| Buffer leaking from inner chamber | Incomplete gasket seal | Wet gasket with running buffer before use |
| | Improper assembly of gel into the electrode/companion assembly | <ul style="list-style-type: none">Ensure that top edge of short plate fits under notch at top of gasketEnsure that top of short plate touches the green gasket |

TIPS

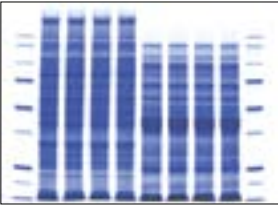


Fig. 1. Schematic of protein migration during SDS-PAGE.

Figure 1 provides an example of an optimal gel image, where the bands are nicely resolved, each lane is very straight, and protein bands are present across the length of the gel (there is excellent separation across the entire molecular weight range).

Electrophoresis (contd.)

| Problem | Cause | Solution |
|--|---------------------------------|--|
| Leaking from upper buffer chamber (Mini-PROTEAN® Tetra cell) | Upper buffer chamber overfilled | Keep buffer level below top of spacer plate |
| | Improper assembly | <ul style="list-style-type: none">• Ensure that U-shaped electrode core gasket is clean, free of cuts, and lubricated with buffer• Ensure that short plate is under notch on gasket |

Total Protein Staining

| Problem | Cause | Solution |
|------------------------------------|--|---|
| Bands not visible | No protein in gel | Stain with another method to confirm there is protein |
| | Imaging system malfunctioning | Check instrument manual for troubleshooting, or contact imaging instrument manufacturer |
| | Incorrect imaging parameters were used | Check instrument manual |
| Poor staining sensitivity | Dirty staining trays | Clean staining trays and other equipment with laboratory glassware cleaner |
| | Insufficient stain volume | Follow recommendations for stain volume (appropriate to gel size) |
| | Insufficient staining time | Increase staining time |
| | Reuse of staining solution | Repeat staining protocol with fresh staining solution |
| High or uneven background staining | Dirty equipment or staining trays | Clean staining trays and other equipment with laboratory glassware cleaner |
| | Too much time in staining solution | <ul style="list-style-type: none">• Restrict duration of incubation in staining solutions as recommended in protocol• Wash gel in water or respective destaining solution for ≥30 min |
| | Reagent impurities | Use high-purity water and reagents for staining |
| Speckles or blotches in gel image | Particulate material from reagents, staining tray, dust, or gloves | <ul style="list-style-type: none">• Clean staining trays thoroughly• Limit time that gels and staining solution are exposed to open air• Use dust-free gloves and handle gels only by edges |
| Uneven staining | Insufficient shaking during staining | Agitate gel during staining |
| Gel shrinkage | Gel dehydrated | Transfer gel to water for rehydration |

Evaluation of Separation

| Problem | Cause | Solution |
|---|--|---|
| Diffuse or broad bands | Poor quality acrylamide or bis-acrylamide, incomplete polymerization | <ul style="list-style-type: none">• Use electrophoresis-grade reagents• Check polymerization conditions |
| | Old SDS or sample buffer | Prepare fresh solutions |
| | Gel temperature too high | Use external cooling during run or run more slowly |
| Bands “smile” across gel, band pattern curves upward at both sides of gel | Excess heating of gel; center of gel runs hotter than either end | <ul style="list-style-type: none">• Check buffer composition; buffer not mixed well, or buffer in upper chamber too concentrated• Prepare new buffer, ensuring thorough mixing, especially when diluting 5x or 10x stock |
| | Power conditions excessive | Do not exceed recommended running conditions. Decrease power power setting from 200 V to 150 V or fill lower chamber to within 1 cm of top of short plate |
| | Insufficient buffer | Fill inner and outer buffer chambers to ensure that wells are completely covered |
| Smiling or frowning bands within gel lane | Overloaded proteins | Load less protein |
| | Sample preparation/buffer issues | Minimize salts, detergents, and solvents in sample preparation and sample buffers |
| | Incorrect running conditions | Use correct voltage |
| Skewed or distorted bands, lateral band spreading | Excess salt in samples | Remove salts from sample by dialysis or desalting column prior to sample preparation |
| | Ionic strength of sample lower than that of gel | Use same buffer in samples as in gel |
| | Insufficient sample buffer or wrong formulation | Check buffer composition and dilution instructions |
| | Diffusion prior to turning on current | Minimize time between sample application and power startup |
| | Diffusion during migration through stacking gel | <ul style="list-style-type: none">• Increase %T of stacking gel to 4.5% or 5% T• Increase current by 25% during stacking |
| | Uneven gel interface | <ul style="list-style-type: none">• Decrease polymerization rate• Overlay gels carefully• Rinse wells after removing comb to remove residual acrylamide |

Evaluation of Separation (contd.)

| Problem | Cause | Solution |
|---|--|--|
| Vertical streaking | Overloaded samples | <ul style="list-style-type: none">• Dilute sample• Selectively remove predominant protein in sample (fractionate)• Reduce voltage by 25% to minimize streaking |
| | Sample precipitation | <ul style="list-style-type: none">• Centrifuge samples to remove particulates prior to sample loading• Dilute sample in sample buffer |
| Fuzzy or spurious artifactual bands | Concentration of reducing agent too low | Use 5% BME or 1% DTT |
| Protein bands do not migrate down as expected | Bands of interest may be neutral or positively charged in buffer used; pI of bands must be ~2 pH units more negative than pH of running buffer | Use SDS-PAGE or a different buffer system in native PAGE or IEF |



Part IV Appendices

Glossary

| | |
|---------------------------|--|
| %C | Cross-linker concentration; weight percentage of cross-linker in a polyacrylamide gel |
| %T | Monomer concentration (acrylamide + cross-linker) in a gel (in g/100 ml). Gels can be made with a single, continuous %T through the gel (single-percentage gels), or with a gradient of %T through the gel (gradient gels) |
| 2-D electrophoresis | Two-dimensional electrophoresis. Proteins are separated first according to isoelectric point (pI) by isoelectric focusing (IEF) and then according to size by SDS-PAGE, yielding a two-dimensional protein map of spots |
| 2-Mercaptoethanol | Reducing agent necessary for cleavage of intra- and inter-molecular disulfide bonds to achieve complete protein unfolding and to maintain proteins in a fully reduced state. Also known as β-mercaptoethanol or BME |
| Acrylamide | Monomer used with a cross-linker to form the matrix used for separating proteins or small DNA molecules |
| Ammonium persulfate (APS) | Initiator used with TEMED (catalyst) to initiate the polymerization of acrylamide and bisacrylamide in making a polyacrylamide gel; (NH ₄) ₂ S ₂ O ₈ |
| Ampholyte | Amphoteric molecule (containing both acidic and basic groups) that exists mostly as a zwitterion in a certain pH range. Ampholytes are used to establish a stable pH gradient for use in isoelectric focusing |
| Anode | Positively charged electrode. Negatively charged molecules (anions) move toward the anode, which is usually colored red |
| Anionic dye | Negatively charged compound used as a stain; used in blotting to stain proteins immobilized on membranes such as nitrocellulose or PVDF |
| Antibody | Immunoglobulin; protein produced in response to an antigen, which specifically binds the portion of the antigen that initiated its production |
| Antigen | Foreign molecule that specifically binds with an antibody |
| Assay | Analysis of the quantity or characteristics of a substance |
| Background | Nonspecific signal or noise that can interfere with the interpretation of valid signals |
| Bio-Spin® columns | Family of Bio-Rad sample preparation products that includes the Bio-Spin 6 and Micro Bio-Spin™ 6 columns used for buffer exchange and desalting applications |
| Bis or bis-acrylamide | A common cross-linker used with acrylamide to form a support matrix; N,N'-methylene-bis-acrylamide |
| Blocking reagent | Protein used to saturate unoccupied binding sites on a blot to prevent nonspecific binding of antibody or protein probes to the membrane |
| Blot | Immobilization of proteins or other molecules onto a membrane, or a membrane that has the molecules adsorbed onto its surface |
| Blue native PAGE | Discontinuous electrophoretic system that allows high-resolution separation of membrane protein complexes in native, enzymatically active states. Membrane protein complexes are solubilized by neutral, nondenaturing detergents like n-dodecyl-β-D-maltoside. After addition of Coomassie (Brilliant) Blue G-250, which binds to the surface of the proteins, separation of the negatively charged complexes according to mass is possible |
| Bromophenol blue | Common tracking dye used to monitor the progress of electrophoresis |
| Carrier ampholytes | Heterogeneous mixture of small (300–1,000 Da) polyamino-polycarboxylate buffering compounds that have closely spaced pI values and high conductivity. Within an electric field, they align according to pI to establish the pH |

| | |
|--------------------------------------|--|
| Cathode | Negatively charged electrode. Positively charged molecules (cations) move toward the cathode, which is usually colored black |
| CHAPS | Zwitterionic detergent (having both positively and negatively charged groups with a net charge of zero) that is widely used for protein solubilization for IEF and 2-D electrophoresis; 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate |
| Comb | Object used to cast wells in an agarose or acrylamide gel. In PAGE applications, square-bottom combs are inserted into the gel sandwich before polymerization to form square-bottomed wells |
| Continuous buffer system | Gel-based electrophoresis system that uses the same buffer (at constant pH) in the gel, sample, and electrode reservoirs. Typically a single-percentage gel is used, and the sample is loaded directly into the part of the gel in which separation occurs. Continuous systems are not common in protein separations; they are used mostly for nucleic acid analysis |
| Coomassie (Brilliant) Blue | Anionic dye used in the total protein staining of gels and blots that comes in two forms: Coomassie (Brilliant) Blue G-250 differs from Coomassie (Brilliant) Blue R-250 by the addition of two methyl groups |
| Criterion™ cells, blotters, and gels | Family of Bio-Rad products used for midi-format vertical electrophoresis; includes the Criterion and Criterion™ Dodeca™ cells, Criterion blotter, and Criterion precast gels |
| Cross-linker | Molecule (for example, bis-acrylamide) used to link polymerizing monomer molecules together to form the gel, a netlike structure. Holes in the nets are called pores, and pore size is determined in part by the cross-linker concentration. Pores may or may not sieve the macromolecules |
| DC™ assay kit | Bio-Rad's detergent-compatible protein assay kit |
| Discontinuous buffer system | Gel-based electrophoresis system that uses different buffers and sometimes different buffer compositions to focus and separate components of a sample. Discontinuous systems typically focus the proteins into tighter bands than continuous gel systems, allowing larger protein loads |
| Dithiothreitol (DTT) | Reducing agent necessary for cleavage of intra- and inter-molecular disulfide bonds to achieve complete protein unfolding and to maintain all proteins in a fully reduced state |
| Electroelution | Technique that applies the principles of electrophoresis to enable recovery (elution) of molecules such as proteins from gels and gel slices |
| Electrophoresis | Movement of charged molecules in a uniform electric field |
| Glycerol | Small nonionic molecule used in vertical gel electrophoresis to increase the density of the sample buffer so that it sinks to the bottom of the sample well; also used to help keep proteins soluble, especially in isoelectric focusing |
| Glycine | Amino acid used as the trailing ion in discontinuous electrophoresis |
| Gradient gel | Gel with gradually changing monomer concentration (%T) in the direction of migration. In SDS-PAGE, gradients are used to separate wider molecular weight ranges than can be separated with single-percentage gels |
| Immobilized pH gradient (IPG) | Strips in which buffering groups are covalently bound to an acrylamide gel matrix, resulting in stable pH gradients except the most alkaline (>12) pH values. This eliminates problems of gradient instability and poor sample loading capacity associated with carrier ampholyte-generated pH gradients |

| | |
|---|--|
| Immunoassay | Test for a substance by its reactivity with an antibody |
| Immunoblotting | Blot detection by antibody binding |
| Immunodetection | Detection of a molecule by its binding to an antibody |
| Immunoglobulin | Antibody; protein produced in response to an antigen, which specifically binds the portion of the antigen that initiated its production |
| Ion front | Group of ions moving together during electrophoresis, marking the movement of the buffer from the upper buffer reservoir. Due to their small size, they are not hindered by a sieving matrix and move together primarily because of their charge |
| Ionic strength | Measure of the ionic concentration of a solution that affects its resistance |
| Isoelectric focusing (IEF) | Electrophoresis technique that separates proteins according to their isoelectric point (pI) |
| Isoelectric point (pI) | pH value at which a molecule carries no electrical charge, or at which the negative and positive charges are equal |
| Ligand | Molecule that binds another in a complex |
| MicroRotor[™] cells and kits | Family of Bio-Rad sample preparation products, including the MicroRotor for liquid-phase IEF cell and MicroRotor for cell lysis kits |
| Monomer | Unit that makes up a polymer (acrylamide is a monomer that is polymerized into polyacrylamide) |
| Mini-PROTEAN[®] cells and gels | Family of Bio-Rad products used for mini-format vertical electrophoresis; includes the Mini-PROTEAN Tetra and Mini-PROTEAN [®] 3 Dodeca [™] cells, and Mini-PROTEAN precast gels |
| Native PAGE | Version of PAGE that retains native protein configuration, performed in the absence of SDS and other denaturing agents |
| Ohm's Law | Describes the mutual dependence of three electrical parameters (V, volts; I, ampere; R, ohm): $V = I \times R$ |
| PAGE | Polyacrylamide gel electrophoresis, a common method of separating proteins |
| Polyacrylamide | Anticonvective sieving matrix used in gel electrophoresis. Polyacrylamide gels are cast using mixtures of acrylamide monomers with a cross-linking reagent, usually N,N'-methylenebisacrylamide (bis), both solubilized in buffer |
| Polyacrylamide gel electrophoresis (PAGE) | Electrophoresis technique that uses polyacrylamide as the separation medium |
| PowerPac[™] power supplies | Family of Bio-Rad power supplies |
| Power supply | Instrument that provides the electric power to drive electrophoresis and electrophoretic blotting experiments |
| Precision Plus Protein[™] standards | Bio-Rad's family of recombinant protein standards |
| Preparative electrophoresis | Electrophoresis techniques that separate large volumes of protein samples (nanogram to gram quantities of protein), generally for the purposes of purification or fractionation (to reduce sample complexity) |
| Primary antibody | Antibody that binds a molecule of interest |
| PROTEAN[®] cells | Family of Bio-Rad products used for large-format vertical electrophoresis; includes the PROTEAN II xi, PROTEAN II XL, and PROTEAN Plus [®] Dodeca [™] cells |

| | |
|-------------------------------------|--|
| Protein standards | Mixtures of well-characterized or recombinant proteins used to monitor separation and estimate the size and concentration of the proteins separated in a gel |
| Prestained standards | Mixture of molecular weight marker proteins that have covalently attached dye molecules, which render the bands visible during electrophoresis and transfer |
| RC DC[™] assay kit | Bio-Rad's reductant- and detergent-compatible protein assay kit |
| Resolving gel | Portion of a discontinuous electrophoresis gel that separates the different bands from each other |
| R_f value | Relative distance a protein has traveled compared to the distance traveled by the ion front. This value is used to compare proteins in different lanes and even in different gels. It can be used with standards to generate standard curves, from which the molecular weight or isoelectric point of an unknown may be determined |
| Running buffer | Buffer that provides the ions for the electrical current in an electrophoresis run. It may also contain denaturing agents. The running buffer provides the trailing ions in discontinuous electrophoresis |
| Sample buffer | Buffer in which a sample is suspended prior to loading onto a gel. SDS-PAGE sample buffer typically contains denaturing agents (including reducing agents and SDS), tracking dye, and glycerol |
| SDS-PAGE | Separation of molecules by molecular weight in a polyacrylamide gel matrix in the presence of a denaturing detergent, sodium dodecyl sulfate (SDS). SDS denatures polypeptides and binds to proteins at a constant charge-to-mass-ratio. In a sieving polyacrylamide gel, the rate at which the resulting SDS-coated proteins migrate in the gel is relative only to their size and not to their charge or shape |
| Secondary antibody | Reporter antibody that binds to a primary antibody; used to facilitate detection |
| Sodium dodecyl sulfate (SDS) | Anionic detergent that denatures proteins and binds to polypeptides in a constant weight ratio of 1:4 (SDS:polypeptide) |
| Spacers | Small blocks set between the two glass plates at the sides of a gel cassette, which create a space between the glass plates in which to pour the slab gel monomer solution |
| Stacking gel | Portion of a discontinuous electrophoresis gel that concentrates the components of the sample to create a very thin starting zone; bands are then separated from each other in the resolving gel |
| Stain-free technology | Protein detection technology involving UV-induced haloalkane modification of protein tryptophan residues. Continued exposure to UV light causes fluorescence of the modified proteins, which are then detected by a CCD imager. Sensitivity of this technique is generally equal to or better than Coomassie staining |
| Stained standards | Mixture of molecular weight marker proteins that have covalently attached dye molecules; the bands are visible during electrophoresis and transfer |
| Standard | Collection of molecules with known properties, such as molecular weight or isoelectric point. Often used to create standard curves, from which properties of an unknown may be determined |
| TEMED | Used with APS (initiator) to catalyze the polymerization of acrylamide and bisacrylamide in making a polyacrylamide gel; N,N,N',N'-tetramethylethylenediamine |
| TGX[™] gels | Bio-Rad's Tris-glycine extended shelf life precast gels |

| | |
|---------------------|---|
| Total protein stain | Reagent that binds nonspecifically to proteins; used to detect the entire protein pattern on a blot or gel |
| Tricine | Organic compound used in SDS-PAGE as a buffer component to replace glycine and improve resolution of small (down to 1–5 kD) proteins |
| Tris | Organic component of buffer solutions that has an effective pH range of 7.0–9.2; tris(hydroxymethyl) aminomethane |
| Transfer | Immobilization of proteins or other molecules onto a membrane by electrophoretic or passive means |
| Triton X-100 | Nonionic detergent widely used for protein solubilization (for IEF and 2-D electrophoresis) |
| Tween 20 | Nonionic detergent; used in blot detection procedures as a blocking reagent or in wash buffers to minimize nonspecific binding and background |
| Unstained standards | Mixture of molecular weight marker proteins that do not have covalently attached dye molecules; the bands are invisible during electrophoresis and transfer, but are useful for molecular weight determination |
| Urea | Chaotrope usually included at rather high concentrations (9.5 M) in sample solubilization buffers for denaturing IEF and 2-D PAGE |
| Western blotting | Immobilization of proteins onto a membrane and subsequent detection by protein-specific binding and detection reagents |
| Zymogram PAGE | Electrophoresis technique used to detect and characterize collagenases and other proteases within the gel. Gels are cast with gelatin or casein, which acts as a substrate for the enzymes that are separated in the gel under nonreducing conditions |

References and Related Reading

References

Sample preparation and protein assay

Berkelman T (2008). Quantitation of protein in samples prepared for 2-D electrophoresis. *Methods Mol Biol* 424, 43–49.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72, 248–254.

Cañas B et al. (2007). Trends in sample preparation for classical and second generation proteomics. *J Chromatogr A* 1153, 235–258.

Chen Y et al. (2008). Sample preparation. *J Chromatogr A* 1184, 191–219.

Damerval C et al. (1988). Two-dimensional electrophoresis in plant biology. *Advances in Electrophoresis* 2, 236–340.

Drews O et al. (2004). Setting up standards and a reference map for the alkaline proteome of the Gram-positive bacterium *Lactococcus lactis*. *Proteomics* 4, 1293–1304.

Evans DR et al. (2009). Concentration of proteins and removal of solutes. *Methods Enzymol* 463, 97–120.

Goldberg S (2008). Mechanical/physical methods of cell disruption and tissue homogenization. *Methods Mol Biol* 424, 3–22.

Harder A et al. (1999). Comparison of yeast cell protein solubilization procedures for two-dimensional electrophoresis. *Electrophoresis* 20, 826–829.

Huber LA et al. (2003). Organelle proteomics: implications for subcellular fractionation in proteomics. *Circ Res.* 92, 962–968.

Lowry OH et al. (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem* 193, 265–275.

Luche S et al. (2003). Evaluation of nonionic and zwitterionic detergents as membrane protein solubilizers in two-dimensional electrophoresis. *Proteomics* 3, 249–253.

Noble JE and Bailey MJ (2009). Quantitation of protein. *Methods Enzymol* 463, 73–95.

Poetsch A and Wolters D (2008). Bacterial membrane proteomics. *Proteomics* 8, 4100–4122.

Posch A et al. (2006). Tools for sample preparation and prefractionation in two-dimensional gel electrophoresis. In *Separation Methods in Proteomics*, Smejkal GB ed. (Boca Raton: CRC Press), 107–133.

Rabilloud T (1996). Solubilization of proteins for electrophoretic analyses. *Electrophoresis* 17, 813–829.

Rhodes DG and Laue TM (2009). Determination of protein purity. *Methods Enzymol* 463, 677–689.

Sapan CV et al. (1999). Colorimetric protein assay techniques. *Biotechnol Appl Biochem* 29, 99–108.

Smith PK et al. (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* 150, 76-85.

Vuillard L et al. (1995). Enhancing protein solubilization with nondetergent sulfobetaines. *Electrophoresis* 16, 295-297.

Electrophoresis

Andrews AT (1986). *Electrophoresis: theory, techniques and biochemical and clinical applications* (New York: Oxford University Press).

Bjellqvist B et al. (1982). Isoelectric focusing in immobilized pH gradients: principle, methodology and some applications. *J Biochem Biophys Methods* 6, 317–339.

Davis BJ (1964). Disc electrophoresis. II. Method and application to human serum proteins. *Ann NY Acad Sci* 121, 404–427.

Dunn MJ (1993). *Gel electrophoresis: Proteins* (Oxford: BIOS Scientific Publishers Ltd.).

Fenselau C (2007). A review of quantitative methods for proteomic studies. *J Chromatogr B Analyt Technol Biomed Life Sci* 855, 14–20.

Garfin DE (1990). One-dimensional gel electrophoresis. *Methods Enzymol* 182, 425–441.

Garfin DE (2009). One-dimensional gel electrophoresis. *Methods Enzymol* 463, 497–513.

Goldenberg DP and Creighton TE (1984). Gel electrophoresis in studies of protein conformation and folding. *Anal Biochem* 138, 1–18.

Hames BD (1998). *Gel electrophoresis of proteins: A practical approach*, 3rd ed. (Oxford: Oxford University Press).

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.

McLellan T (1982). Electrophoresis buffers for polyacrylamide gels at various pH. *Anal Biochem* 126, 94–99.

Niepmann M (2007). Discontinuous native protein gel electrophoresis: pros and cons. *Expert Rev Proteomics* 4, 355–361.

Nijtmans LG et al. (2002). Blue Native electrophoresis to study mitochondrial and other protein complexes. *Methods* 26, 327–334.

O'Farrell PH (1975). High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250, 4007–4021.

Ornstein L (1964). Disc electrophoresis I: background and theory. *Ann NY Acad Sci* 121, 321–349.

Rabilloud T (2010). Variations on a theme: changes to electrophoretic separations that can make a difference. *J Proteomics* 73, 1562–1572.

Reisinger V and Eichacker LA (2008). Isolation of membrane protein complexes by blue native electrophoresis. *Methods Mol Biol* 424, 423–431.

Schägger H and von Jagow G (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 166, 368–379.

Vavricka SR et al. (2009). Serum protein electrophoresis: an underused but very useful test. *Digestion* 79, 203–210.

Westermeier R (2004). Isoelectric focusing. *Methods Mol Biol* 244, 225–232.

Wheeler D et al. (2004). Discontinuous buffer systems operative at pH 2.5 - 11.0, 0 degrees C and 25 degrees C, available on the Internet. *Electrophoresis* 25, 973–974.

Zewert TE and Harrington MG (1993). Protein electrophoresis. *Curr Opin Biotechnol* 4, 3–8.

Staining

Agrawal GK and Thelen JJ (2009). A high-resolution two dimensional Gel- and Pro-Q DPS-based proteomics workflow for phosphoprotein identification and quantitative profiling. *Methods Mol Biol* 527, 3–19.

Fernandez-Patron C et al. (1992). Reverse staining of sodium dodecyl sulfate polyacrylamide gels by imidazole-zinc salts: sensitive detection of unmodified proteins. *Biotechniques* 12, 564-573.

Gottlieb M and Chavko M (1987). Silver staining of native and denatured eucaryotic DNA in agarose gels. *Anal Biochem* 165, 33-37.

Hart C et al. (2003). Detection of glycoproteins in polyacrylamide gels and on electroblobs using Pro-Q Emerald 488 dye, a fluorescent periodate Schiff-base stain. *Electrophoresis* 24, 588–598.

Lee C et al. (1987). Copper staining: a five-minute protein stain for sodium dodecyl sulfate-polyacrylamide gels. *Anal Biochem* 166, 308-312.

Merril CR (1987). Detection of proteins separated by electrophoresis. *Adv Electrophoresis* 1, 111–139.

Merril CR et al. (1981). Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* 211, 1437–1438.

Miller I et al. (2006). Protein stains for proteomic applications: which, when, why? *Proteomics*, 6, 5385–5408.

Neuhoff V et al. (1988). Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. *Electrophoresis* 9, 255–262.

Oakley BR et al. (1980). A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal Biochem* 105, 361–363.

Rabilloud T et al. (1994). Silver-staining of proteins in polyacrylamide gels: a general overview. *Cell Mol Biol* 40, 57–75.

Simpson RJ (2010). Rapid coomassie blue staining of protein gels. *Cold Spring Harb Protoc*, pdb prot5413.

Sinha P et al. (2001). A new silver staining apparatus and procedure for matrix-assisted laser desorption/ionization-time of flight analysis of proteins after two-dimensional electrophoresis. *Proteomics* 1, 835-840.

Steinberg TH (2009). Protein gel staining methods: an introduction and overview. *Methods Enzymol* 463, 541–563.

Steinberg TH et al. (2003). Global quantitative phosphoprotein analysis using multiplexed proteomics technology. *Proteomics* 3, 1128–1144.

Westermeier R and Marouga R (2005). Protein detection methods in proteomics research. *Biosci Rep* 25, 19–32.

Yan JX et al. (2000). A modified silver staining protocol for visualization of proteins compatible with matrix-assisted laser desorption/ionization and electrospray ionization-mass spectrometry. *Electrophoresis* 21, 3666–3672.

Bio-Rad Bulletins

Technical Notes

| Bulletin | Title |
|----------|---|
| 6387 | Biologics Analysis Workflow Comparability Study |
| 2651 | 2-D Electrophoresis for Proteomics: A Methods and Product Manual |
| 2895 | Protein Blotting Guide, A Guide to Transfer and Detection |
| 1156 | Acrylamide Polymerization – a Practical Approach |
| 1909 | Modification of Bio-Rad DC™ Protein Assay for Use with Thiols |
| 6001 | Rapid Validation of Purified Proteins Using Criterion™ Stain Free™ Gels |
| 5910 | Mini-PROTEAN® TGX™ Precast Gel: A Gel for SDS-PAGE with Improved Stability — Comparison with Standard Laemmli Gels |
| 5911 | Mini-PROTEAN TGX Precast Gel: A Versatile and Robust Laemmli-Like Precast Gel for SDS-PAGE |
| 5932 | Ready Gel® to Mini-PROTEAN TGX Precast Gels Catalog Number Conversion Chart |
| 5934 | NuPAGE Bis-Tris Precast Gels (MOPS Buffer) to Mini-PROTEAN TGX Precast Gels Catalog Number Conversion Chart |
| 3133 | Molecular Weight Determination by SDS-PAGE |
| 3144 | Using Precision Plus Protein™ Standards to Determine Molecular Weight |
| 5956 | Precision Plus Protein Dual Xtra Standards – New Protein Standards with an Extended Range from 2 to 250 kD |
| 5763 | Molecular Weight Estimation Using Precision Plus Protein™ WesternC™ Standards on Criterion Tris-HCl and Criterion™ XT Bis-Tris Gels |
| 5576 | Molecular Weight Estimation and Quantitation of Protein Samples Using Precision Plus Protein WesternC Standards, the Immun-Star™ WesternC™ Chemiluminescent Detection Kit, and the Molecular Imager® ChemiDoc™ XRS Imaging System |
| 2043 | Purification of Proteins from <i>Mycobacterium tuberculosis</i> |
| 2168 | Isolation of Hydrophobic <i>C. albicans</i> Cell Wall Protein by In-Line Transfer From Continuous Elution Preparative Electrophoresis |
| 2376 | Gel Electrophoresis: Separation of Native Basic Proteins by Cathodic, Discontinuous Polyacrylamide Gel Electrophoresis |
| 5705 | Sensitivity and Protein-to-Protein Consistency of Flamingo™ Fluorescent Gel Stain Compared to Other Fluorescent Stains |
| 5754 | Comparison of SYPRO Ruby and Flamingo Fluorescent Gel Stains with Respect to Compatibility with Mass Spectrometry |
| 5921 | Oriole™ Fluorescent Gel Stain: Characterization and Comparison with SYPRO Ruby Gel Stain |
| 5989 | Imaging Fluorescently Stained Gels with Image lab™ Software |
| 5723 | Increase Western Blot Throughput with Multiplex Fluorescent Detection |
| 5294 | MicroRotofor Cell Product Information Sheet |

Product Information

| Bulletin | Title |
|----------|---|
| 6385 | Biologics Analysis Workflow Brochure |
| 5517 | MicroRotofor™ Lysis Kits Flier |
| 1069 | Colorimetric Protein Assays |
| 2826 | SmartSpec™ Plus Spectrophotometer Brochure |
| 2414 | The Little Book of Standards |
| 2998 | Protein Standards Application Guide |
| 2317 | Ready-to-Run Buffers and Solutions Brochure |
| 5535 | Mini-PROTEAN® Tetra Cell Brochure |
| 5871 | Mini-PROTEAN® TGX™ Precast Gels Product Information Sheet |
| 2710 | Criterion™ Precast Gel System Brochure |
| 2911 | Criterion XT Precast Gels Product Information Sheet |
| 5974 | Criterion TGX Stain-Free Precast Gels Product Information Sheet |
| 1760 | PROTEAN® II xi and XL Cells Product Information Sheet |
| 6371 | Electrophoresis Power Supplies Brochure |
| 2423 | Bio-Safe™ Coomassie Stain Brochure |
| 5346 | Flamingo™ Fluorescent Gel Stain Product Information Sheet |
| 5900 | Oriole™ Fluorescent Gel Stain Product Information Sheet |
| 5888 | Bio-Rad Imaging Systems Family Brochure |
| 3096 | Expression Proteomics Brochure |
| 2032 | Western Blotting Detection Reagents Brochure |
| 5294 | MicroRotofor Cell Product Information Sheet |

Instruction Manuals

| Bulletin | Title |
|------------|---|
| 10007296 | Mini-PROTEAN Tetra Cell |
| 1658100 | Mini-PROTEAN Precast Gels |
| 4006183 | Criterion Cell |
| 4006213 | PowerPac Basic Power Supply |
| 4006222 | PowerPac HC Power Supply |
| 4006223 | PowerPac Universal Power Supply |
| 4110001 | Criterion Gel Application Guide |
| 4110130 | Criterion XT Precast Gels |
| 4110065 | Quick Start™ Bradford Protein Assay |
| 4110107 | RC DC™ Protein Assay |
| 4000126-14 | The Discovery Series: Quantity One® 1-D Analysis Software |
| LIT33 | Bio-Rad Protein Assay |
| LIT448 | DC Protein Assay |

Ordering Information

Electrophoresis Instrumentation

| Catalog # | Description |
|--|---|
| Mini-PROTEAN® Tetra Cells and Systems | |
| 165-8000 | Mini-PROTEAN Tetra Cell , 10-well, 0.75 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam |
| 165-8001 | Mini-PROTEAN Tetra Cell , 10-well, 1.0 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam |
| 165-8002 | Mini-PROTEAN Tetra Cell , 2 10-well, 0.75 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam |
| 165-8003 | Mini-PROTEAN Tetra Cell , 10-well, 1.0 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam |
| 165-8004 | Mini-PROTEAN Tetra Cell for Mini Precast Gels , 4-gel system includes electrode assembly, clamping frame, companion module, tank, lid with power cables, mini cell buffer dam |
| 165-8005 | Mini-PROTEAN Tetra Cell for Mini Precast Gels , 2-gel system includes electrode assembly, clamping frame, tank, lid with power cables, mini cell buffer dam |
| 165-8006 | Mini-PROTEAN Tetra Cell , 10-well, 1.5 mm thickness; 4-gel system includes 5 combs, 5 sets of glass plates, 2 casting stands, 4 casting frames, sample loading guide, electrode assembly, companion running module, tank, lid with power cables, mini cell buffer dam |
| 165-8007 | Mini-PROTEAN Tetra Cell , 10-well, 1.5 mm thickness; 2-gel system includes 5 combs, 5 sets of glass plates, casting stand, 2 casting frames, sample loading guide, electrode assembly, tank, lid with power cables, mini cell buffer dam |
| 165-8025 | Mini-PROTEAN Tetra Cell and PowerPac™ Basic Power Supply , includes 165-8001 and 164-5050 |
| 165-8026 | Mini-PROTEAN Tetra Cell and PowerPac™ Universal Power Supply , includes 165-8001 and 164-5070 |
| 165-8027 | Mini-PROTEAN Tetra Cell and PowerPac™ HC Power Supply , includes 165-8001 and 164-5052 |
| 165-8028 | Mini-PROTEAN Tetra Cell and PowerPac™ HV Power Supply , includes 165-8001 and 164-5056 |
| 165-8029 | Mini-PROTEAN Tetra Cell and Mini Trans-Blot® Module , includes 165-8001 and 170-3935 |
| 165-8030 | Mini-PROTEAN Tetra Cell for Mini Precast Gels and Mini Trans-Blot Module , includes 165-8004 and 170-3935 |








| Catalog # | Description |
|--|--|
| 165-8033 | Mini-PROTEAN Tetra Cell, Mini Trans-Blot Module, and PowerPac Basic Power Supply , includes 165-8001, 170-3935, and 164-5050 |
| 165-8034 | Mini-PROTEAN Tetra Cell for Mini Precast Gels, Mini Trans-Blot Module, and PowerPac Basic Power Supply , includes 165-8004, 170-3935, and 164-5050 |
| 165-8035 | Mini-PROTEAN Tetra Cell, Mini Trans-Blot Module, and PowerPac HC Power Supply , includes 165-8001, 170-3935, and 164-5052 |
| 165-8036 | Mini-PROTEAN Tetra Cell for Mini Precast Gels, Mini Trans-Blot Module, and PowerPac HC Power Supply , includes 165-8004, 170-3935, and 164-5052 |
| Mini-PROTEAN® Dodeca™ Cells and Systems | |
| 165-4100 | Mini-PROTEAN 3 Dodeca Cell , includes electrophoresis tank with built-in cooling coil, lid with power cables, 6 electrophoresis clamping frames, 2 buffer dams, drain line, 2 gel releasers |
| 165-4101 | Mini-PROTEAN 3 Dodeca Cell with Multi-Casting Chamber , same as 165-4100 with multi-casting chamber, 15 separation sheets, 8 acrylic blocks, tapered luer connector, stopcock valve |
| Criterion™ Cells and Systems | |
| 165-6001 | Criterion Cell , includes buffer tank, lid with power cables, 3 sample loading guides (12 + 2-well, 18-well, 26-well) |
| 165-6019 | Criterion Cell and PowerPac Basic Power Supply , 100–120/220–240 V, includes 165-6001 and 164-5050 |
| Criterion™ Dodeca™ Cells and Systems | |
| 165-4130 | Criterion Dodeca Cell , includes electrophoresis buffer tank with built-in cooling coil, lid with power cables |
| 165-4138 | Criterion Dodeca Cell and PowerPac HC Power Supply , includes 165-4130 and 164-5052 |
| 165-4139 | Criterion Dodeca Cell and PowerPac Universal Power Supply , includes 165-4130 and 164-5070 |
| 165-5133 | Criterion Dodeca Cell and 6-Row AnyGel™ Stand , includes 165-4130 and 165-5131 |
| PROTEAN® II xi Cells | |
| 165-1801 | PROTEAN II xi Cell , 16 cm, without spacers and combs |
| 165-1802 | PROTEAN II xi Cell , 16 cm, 1.5 mm spacers (4), 15-well combs (2) |
| 165-1803 | PROTEAN II xi Cell , 16 cm, 1.0 mm spacers (4), 15-well combs (2) |
| 165-1804 | PROTEAN II xi Cell , 16 cm, 0.75 mm spacers (4), 15-well combs (2) |
| 165-1811 | PROTEAN II xi Cell , 20 cm, without spacers and combs |
| 165-1812 | PROTEAN II xi Cell , 20 cm, 1.5 mm spacers (4), 15-well combs (2) |
| 165-1813 | PROTEAN II xi Cell , 20 cm, 1.0 mm spacers (4), 15-well combs (2) |
| 165-1814 | PROTEAN II xi Cell , 20 cm, 0.75 mm spacers (4), 15-well combs (2) |

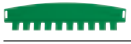
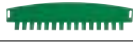



| Catalog # | Description |
|--|---|
| PROTEAN® Plus Dodeca™ Cells and Systems | |
| 165-4150 | PROTEAN Plus Dodeca Cell , 100/120 V, includes electrophoresis buffer tank with built-in ceramic cooling core, lid, buffer recirculation pump with tubing, 2 gel releasers |
| 165-4140 | PROTEAN Plus Dodeca Cell (100/120 V) and PowerPac HC Power Supply , includes 165-4150 and 164-5052 |
| 165-4142 | PROTEAN Plus Dodeca Cell (100/120 V) and PowerPac Universal Power Supply , includes 165-4150 and 164-5070 |
| 165-4144 | PROTEAN Plus Dodeca Cell (100/120 V) , Trans-Blot Plus Cell, and PowerPac Universal Power Supply, includes 165-4150, 170-3990, and 164-5070 |
| 165-5134 | PROTEAN Plus Dodeca Cell (100/120 V) and Two 6-Row AnyGel Stands , includes 165-4150 and two 165-5131 |
| 165-4151 | PROTEAN Plus Dodeca Cell , 220/240 V, includes electrophoresis buffer tank with built-in ceramic cooling core, lid, buffer recirculation pump with tubing, 2 gel releasers |
| 165-4141 | PROTEAN Plus Dodeca Cell (220/240 V) and PowerPac HC Power Supply , includes 165-4151 and 164-5052 |
| 165-4143 | PROTEAN Plus Dodeca Cell (220/240 V) and PowerPac Universal Power Supply , includes 165-4151 and 164-5070 |
| 165-4145 | PROTEAN Plus Dodeca Cell (220/240 V) , Trans-Blot Plus Cell, and PowerPac Universal Power Supply, includes 165-4151, 170-3990, and 164-5070 |
| 165-5135 | PROTEAN Plus Dodeca Cell (220/240 V) and Two 6-Row AnyGel Stands , includes 165-4151 and two 165-5131 |
| Power Supplies | |
| 164-5050 | PowerPac Basic Power Supply , 100–120/220–240 V |
| 164-5052 | PowerPac HC Power Supply , 100–120/220–240 V |
| 164-5056 | PowerPac HV Power Supply , 100–120/220–240 V |
| 164-5070 | PowerPac Universal Power Supply , 100–120/220–240 V |
| Sample Preparation Kits | |
| 163-2141 | MicroRotorfor™ Cell Lysis Kit (Mammal) , 15 preps, includes 50 ml protein solubilization buffer (PSB), ReadyPrep™ mini grinders (2 packs of 10 each) |
| 163-2142 | MicroRotorfor Cell Lysis Kit (Plant) , 10 preps, includes 50 ml protein solubilization buffer (PSB), ReadyPrep 2-D cleanup kit (50 reaction size) |
| 163-2143 | MicroRotorfor Cell Lysis Kit (Yeast) , 15 preps, includes 50 ml protein solubilization buffer (PSB), 15 ml yeast suspension buffer, 2 x 0.5 ml lyticase (1.5 U/μl) |
| 163-2144 | MicroRotorfor Cell Lysis Kit (Bacteria) , 15 preps, includes 50 ml protein solubilization buffer (PSB), 25 ml bacteria suspension buffer, 1 ml lysozyme (1,500 U/μl) |
| 163-2140 | ReadyPrep 2-D Cleanup Kit , 5 preps |
| 732-6221 | Micro Bio-Spin™ 6 Columns , includes 25 columns in Tris buffer, 50 collection tubes |
| 732-6227 | Bio-Spin® 6 Columns , includes 25 columns in Tris buffer, 50 collection tubes |
| 732-6228 | Bio-Spin 6 Columns , includes 100 columns in Tris buffer, 200 collection tubes |

| Catalog # | Description |
|--|---|
| Protein Assay Kits and Instruments | |
| 500-0001 | Bio-Rad Protein Assay Kit I , includes 450 ml dye reagent concentrate, bovine β-globulin standard; sufficient for 440 standard assays or 2,200 microplate assays |
| 500-0002 | Bio-Rad Protein Assay Kit II , includes 450 ml dye reagent concentrate, bovine serum albumin standard; sufficient for 440 standard assays or 2,200 microplate assays |
| 500-0111 | DC™ Protein Assay Kit I , includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution, bovine β-globulin standard; sufficient for 450 standard assays |
| 500-0112 | DC Protein Assay Kit II , includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution, bovine serum albumin standard; sufficient for 450 standard assays |
| 500-0120 | RC DC™ Protein Assay Reagents Package , includes 250 ml alkaline copper tartrate solution, 2 L dilute Folin reagent, 5 ml surfactant solution; sufficient for 450 standard assays |
| 500-0121 | RC DC Protein Assay Kit I , includes RC reagents package, DC reagents package, bovine β-globulin standard; sufficient for 450 standard assays |
| 500-0122 | RC DC Protein Assay Kit II , includes RC reagents package, DC reagents package, bovine serum albumin standard; sufficient for 450 standard assays |
| 500-0201 | Quick Start™ Bradford Protein Assay Kit 1 , includes 1x dye reagent (1 L), bovine serum albumin standard (5 x 2 mg/ml); sufficient for 200 standard assays or 4,000 microplate assays |
| 500-0202 | Quick Start Bradford Protein Assay Kit 2 , includes 1x dye reagent (1 L), bovine serum albumin standard set (2 sets of 7 concentration standards, 0.125–2.0 mg/ml, 2 ml) |
| 500-0203 | Quick Start Bradford Protein Assay Kit 3 , includes 1x dye reagent (1 L), bovine β-globulin standard (5 x 2 mg/ml) |
| 500-0204 | Quick Start Bradford Protein Assay Kit 4 , includes 1x dye reagent (1 L), bovine β-globulin standard set (2 sets of 7 concentration standards, 0.125–2.0 mg/ml, 2 ml) |
| 170-2525 | SmartSpec™ Plus Spectrophotometer |
| 170-2502 | Standard Cuvette , 1–3.5 ml, quartz |
| 170-2511 | trUView™ Cuvettes , pack of 100, individually packaged, disposable DNase- and RNase-free cuvettes |
| Sample Preparation Buffers and Reagents | |
| 161-0747 | 4x Laemmli Sample Buffer , 10 ml |
| 161-0737 | Laemmli Sample Buffer , 30 ml |
| 161-0738 | Native Sample Buffer , 30 ml |
| 161-0739 | Tricine Sample Buffer , 30 ml |
| 161-0763 | IEF Sample Buffer , 30 ml |
| 161-0764 | Zymogram Sample Buffer , 30 ml |
| 161-0791 | XT Sample Buffer , 4x, 10 ml |
| 161-0792 | XR Reducing Agent , 1 ml |
| 161-0719 | Tris , 1 kg |
| 161-0718 | Glycine , 1 kg |
| 161-0301 | SDS , 100 g |
| 161-0416 | SDS Solution , 10% (w/v), 250 ml |
| 166-2404 | 10% Tween 20 , 5 ml |
| 161-0710 | 2-Mercaptoethanol , 25 ml |
| 161-0611 | Dithiothreitol , 5 g |
| 161-0404 | Bromophenol Blue , 10 g |
| 161-0730 | Urea , 250 g |






| Catalog # | Description | Catalog # | Description |
|--|--|---|--|
| Protein Standards | | Running Buffers and Reagents | |
| Recombinant Prestained Protein Standards | | | |
| 161-0393 | Precision Plus Protein All Blue Standards Value Pack , 5 x 500 µl | 161-0732 | 10x Tris/Glycine/SDS , 1 L |
| 161-0373 | Precision Plus Protein All Blue Standards , 500 µl | 161-0734 | 10x Tris/Glycine , 1 L |
| 161-0394 | Precision Plus Protein Dual Color Standards Value Pack , 5 x 500 µl | 161-0744 | 10x Tris/Tricine/SDS , 1 L |
| 161-0374 | Precision Plus Protein Dual Color Standards , 500 µl | 161-0788 | XT MOPS Running Buffer , 20x, 500 ml |
| 161-0397 | Precision Plus Protein Dual Xtra Standards Value Pack , 5 x 500 µl | 161-0789 | XT MES Running Buffer , 20x, 500 ml |
| 161-0377 | Precision Plus Protein Dual Xtra Standards , 500 µl | 161-0790 | XT Tricine Running Buffer , 20x, 500 ml |
| 161-0395 | Precision Plus Protein Kaleidoscope Standards Value Pack , 5 x 500 µl | 161-0793 | XT MOPS Buffer Kit , includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent |
| 161-0375 | Precision Plus Protein Kaleidoscope Standards , 500 µl | 161-0796 | XT MES Buffer Kit , includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent |
| 161-0399 | Precision Plus Protein WesternC Standards Value Pack , 5 x 250 µl | 161-0797 | XT Tricine Buffer Kit , includes 500 ml 20x XT MOPS running buffer, 10 ml 4x XT sample buffer, 1 ml 20x XT reducing agent |
| 161-0376 | Precision Plus Protein WesternC Standards , 250 µl | 161-0761 | 10x IEF Anode Buffer , 250 ml |
| 161-0398 | Precision Plus Protein WesternC (Standards + HRP) Value Pack , 5 x 250 µl | 161-0762 | 10x IEF Cathode Buffer , 250 ml |
| 161-0385 | Precision Plus Protein WesternC (Standards + HRP) , 250 µl | 161-0765 | Zymogram Renaturation Buffer , 125 ml |
| Recombinant Unstained Protein Standards | | 161-0766 | Zymogram Development Buffer , 125 ml |
| 161-0396 | Precision Plus Protein Unstained Standards Value Pack , 5 x 1000 µl | 161-0729 | EDTA , 500 g |
| 161-0363 | Precision Plus Protein Unstained Standards , 1000 µl | 161-0718 | Glycine , 1 kg |
| Natural Prestained SDS-PAGE Protein Standards | | 161-0713 | Tricine , 500 g |
| 161-0324 | Kaleidoscope Prestained Standards , 500 µl | 161-0719 | Tris , 1 kg |
| 161-0305 | Prestained SDS-PAGE Standards , low range, 500 µl | Gel Casting Buffers and Reagents | |
| 161-0309 | Prestained SDS-PAGE Standards , high range, 500 µl | 161-5100 | SDS-PAGE Reagent Starter Kit , includes 100 g acrylamide, 5 g bis, 5 ml TEMED, 10 g ammonium persulfate |
| 161-0318 | Prestained SDS-PAGE Standards , broad range, 500 µl | 161-0100 | Acrylamide , 99.9%, 100 g |
| Natural Unstained SDS-PAGE Protein Standards | | 161-0120 | Acrylamide/Bis Powder , 19:1, 30 g |
| 161-0303 | SDS-PAGE Standards , high range, 200 µl | 161-0122 | Acrylamide/Bis Powder , 37.5:1, 30 g |
| 161-0304 | SDS-PAGE Standards , low range, 200 µl | 161-0140 | 40% Acrylamide Solution , 500 ml |
| 161-0317 | SDS-PAGE Standards , broad range, 200 µl | 161-0144 | 40% Acrylamide/Bis Solution , 19:1, 500 ml |
| 161-0326 | Polypeptide SDS-PAGE Standards , 200 µl | 161-0146 | 40% Acrylamide/Bis Solution , 29:1, 500 ml |
| | | 161-0148 | 40% Acrylamide/Bis Solution , 37.5:1, 500 ml |
| | | 161-0154 | 30% Acrylamide/Bis Solution , 19:1, 500 ml |
| | | 161-0156 | 30% Acrylamide/Bis Solution , 29:1, 500 ml |
| | | 161-0158 | 30% Acrylamide/Bis Solution , 37.5:1, 500 ml |
| | | 161-0200 | Bis Crosslinker , 5 g |
| | | 161-0800 | TEMED , 5 ml |
| | | 161-0798 | Resolving Gel Buffer , 1.5 M tris-HCl, pH 8.8, 1 L |
| | | 161-0700 | Ammonium Persulfate (APS) , 10 g |
| | | 161-0799 | Stacking Gel Buffer , 0.5 M tris-HCl, pH 6.8, 1 L |

Precast Gels

| Description |  |  |  |  |  |  |  |
|---------------------------------|---|---|---|---|---|---|---|
| | 10-Well 30 µl | 15-Well 15 µl | Prep+1-Well 450 µl | 10-Well 50 µl | 12-Well* 20 µl | 9-Well* 30 µl | IPG Well 7 cm IPG Strip |
| Ready Gel® Tris-HCl Gels | | | | | | | |
| 5% Resolving Gel | 161-1210 | 161-1211** | — | 161-1213 | 161-1214** | — | — |
| 7.5% Resolving Gel | 161-1100 | 161-1118 | 161-1136** | 161-1154 | 161-1172 | — | — |
| 10% Resolving Gel | 161-1101 | 161-1119 | 161-1137 | 161-1155 | 161-1173 | 161-1191** | 161-1390** |
| 12% Resolving Gel | 161-1102 | 161-1120 | 161-1138 | 161-1156 | 161-1174 | — | 161-1391 |
| 15% Resolving Gel | 161-1103 | 161-1121 | 161-1139** | 161-1157 | 161-1175 | — | — |
| 18% Resolving Gel | 161-1216 | 161-1217** | — | 161-1219 | 161-1220** | — | — |
| 4–15% Linear Gradient | 161-1104 | 161-1122 | 161-1140 | 161-1158 | 161-1176 | 161-1194** | 161-1392** |
| 4–20% Linear Gradient | 161-1105 | 161-1123 | 161-1141 | 161-1159 | 161-1177 | — | 161-1393** |
| 8–16% Linear Gradient | 161-1222 | 161-1223 | — | 161-1225 | 161-1226 | — | 161-1394 |
| 10–20% Linear Gradient | 161-1106 | 161-1124 | 161-1142** | 161-1160 | 161-1178 | — | 161-1395** |
| Ready Gel IEF Gels | | | | | | | |
| pH 3–10 | 161-1111 | 161-1129** | — | 161-1165** | — | — | — |
| pH 5–8 | 161-1112** | — | — | — | — | — | — |
| Ready Gel Zymogram Gels | | | | | | | |
| 10% Zymogram Gel with Gelatin | 161-1113 | 161-1131** | — | 161-1167 | 161-1185** | — | — |
| 12% Zymogram Gel with Casein | 161-1114** | — | — | 161-1168** | — | — | — |

| Description |  | |  |  |  |  |
|---|---|------------------|---|---|---|---|
| | 10-Well 30 µl | 10-Well 50 µl | 15-Well 15 µl | IPG Well 7cm IPG Strip | 12-Well 20 µl | 8+1-Well 30 µl |
| 10-Gels/Box | | | | | | |
| Mini-PROTEAN® TGX™ Resolving Gels | | | | | | |
| 7.5% | 456-1023 | 456-1024 | 456-1026 | 456-1021 | 456-1025 | 456-1029 |
| 10% | 456-1033 | 456-1034 | 456-1036 | 456-1031 | 456-1035 | 456-1039 |
| 12% | 456-1043 | 456-1044 | 456-1046 | 456-1041 | 456-1045 | 456-1049 |
| 18% | 456-1073 | 456-1074 | 456-1076 | 456-1071 | 456-1075 | 456-1079 |
| 4–15% | 456-1083 | 456-1084 | 456-1086 | 456-1081 | 456-1085 | 456-1089 |
| 4–20% | 456-1093 | 456-1094 | 456-1096 | 456-1091 | 456-1095 | 456-1099 |
| 8–16% | 456-1103 | 456-1104 | 456-1106 | 456-1101 | 456-1105 | 456-1109 |
| 10–20% | 456-1113 | 456-1114 | 456-1116 | 456-1111 | 456-1115 | 456-1119 |
| Any kD | 456-9033 | 456-9034 | 456-9036 | 456-9031 | 456-9035 | 456-9039 |
| Mini-PROTEAN® TGX Stain-Free™ Gels | | | | | | |
| 7.5% | 456-8023 | 456-8024 | 456-8026 | 456-8021 | 456-8025 | 456-8029 |
| 10% | 456-8033 | 456-8034 | 456-8036 | 456-8031 | 456-8035 | 456-8039 |
| 12% | 456-8043 | 456-8044 | 456-8046 | 456-8041 | 456-8045 | 456-8049 |
| 4–15% | 456-8083 | 456-8084 | 456-8086 | 456-8081 | 456-8085 | 456-8089 |
| 4–20% | 456-8093 | 456-8094 | 456-8096 | 456-8091 | 456-8095 | 456-8099 |
| 8–16% | 456-8103 | 456-8104 | 456-8106 | 456-8101 | 456-8105 | 456-8109 |
| Any kD | 456-8123 | 456-8124 | 456-8126 | 456-8121 | 456-8125 | 456-8129 |
| Mini-PROTEAN Tris-Tricine Gels (Pack of 2) | | | | | | |
| 16.5% Resolving Gel | 456-3063 | 456-3064 | 456-3066 | — | 456-3065* | |
| 10–20% Resolving Gel | 456-3113 | 456-3114 | 456-3116* | — | 456-3115* | |
| Mini-PROTEAN TBE Gels (Pack of 2) | | | | | | |
| 5% TBE Gel | 456-5013 | 456-5014* | 456-5016 | — | 456-5015* | |
| 10% TBE Gel | 456-5033 | 456-5034* | 456-5036 | — | 456-5035 | |
| 15% TBE Gel | 456-5053* | 456-5054 | 456-5056 | — | 456-5055* | |
| 4–20% TBE Gel | 456-5093* | 456-5094* | 456-5096* | — | 456-5095* | |
| Mini-PROTEAN TBE-Urea Gels (Pack of 2) | | | | | | |
| 10% TBE-Urea Gel | 456-6033* | — | 456-6036* | — | — | |
| 15% TBE-Urea Gel | 456-6053* | — | 456-6056* | — | 456-6055* | |

All formats are available as both ten packs (catalog numbers listed) and two packs. To order as a two pack, add an "S" to the end of the catalog number for the corresponding ten pack.

| |  12+2-Well** 45 µl |  18-Well 30 µl |  26-Well* 15 µl |  Prep+2-Well** 800 µl |  IPG+1-Well** 11 cm IPG Strip |
|--|---|---|---|--|--|
| Description | | | | | |
| Criterion™ TGX™ Gels** | | | | | |
| 7.5% | 567-1023 | 567-1024 | 567-1025 | — | — |
| 10% | 567-1033 | 567-1034 | 567-1035 | — | — |
| 12% | 567-1043 | 567-1044 | 567-1045 | — | — |
| 18% | 567-1073 | 567-1074 | 567-1075 | 567-1072 | 567-1071 |
| 4–15% | 567-1083 | 567-1084 | 567-1085 | 567-1082 | 567-1081 |
| 4–20% | 567-1093 | 567-1094 | 567-1095 | 567-1092 | 567-1091 |
| 8–16% | 567-1103 | 567-1104 | 567-1105 | 567-1102 | 567-1101 |
| 10–20% | 567-1113 | 567-1114 | 567-1115 | 567-1112 | 567-1111 |
| Any kD | 567-1123 | 567-1124 | 567-1125 | 567-1122 | 567-1121 |
| Criterion™ TGX Stain-Free™ Gels** | | | | | |
| 7.5% | 567-8023 | 567-8024 | 567-8025 | — | — |
| 10% | 567-8033 | 567-8034 | 567-8035 | — | — |
| 12% | 567-8043 | 567-8044 | 567-8045 | — | — |
| 18% | 567-8073 | 567-8074 | 567-8075 | 567-8072 | 567-8071 |
| 4–15% | 567-8083 | 567-8084 | 567-8085 | 567-8082 | 567-8081 |
| 4–20% | 567-8093 | 567-8094 | 567-8095 | 567-8092 | 567-8091 |
| 8–16% | 567-8103 | 567-8104 | 567-8105 | 567-8102 | 567-8101 |
| 10–20% | 567-8113 | 567-8114 | 567-8115 | 567-8112 | 567-8111 |
| Any kD | 567-8123 | 567-8124 | 567-8125 | 567-8122 | 567-8121 |
| Criterion XT Bis-Tris Gels*** | | | | | |
| 10% Resolving Gel | 345-0111 | 345-0112 | 345-0113 | — | 345-0115 |
| 12% Resolving Gel | 345-0117 | 345-0118 | 345-0119 | 345-0120† | 345-0121 |
| 4–12% Resolving Gel | 345-0123 | 345-0124 | 345-0125 | 345-0126† | 345-0127 |
| Criterion XT Tris-Acetate Gels | | | | | |
| 7% Resolving Gel | 345-0135 | 345-0136† | 345-0137† | — | — |
| 3–8% Resolving Gel | 345-0129 | 345-0130 | 345-0131 | — | 345-0133† |
| Criterion Tris-HCl Gels | | | | | |
| 5% Resolving Gel | 345-0001 | 345-0002 | 345-0003† | — | — |
| 7.5% Resolving Gel | 345-0005 | 345-0006 | 345-0007 | 345-0008 | — |
| 10% Resolving Gel | 345-0009 | 345-0010 | 345-0011 | 345-0012† | 345-0101 |
| 12.5% Resolving Gel | 345-0014 | 345-0015 | 345-0016 | 345-0017† | 345-0102 |
| 15% Resolving Gel | 345-0019 | 345-0020 | 345-0021 | 345-0022† | — |
| 18% Resolving Gel | 345-0023 | 345-0024 | 345-0025 | 345-0026† | — |
| 4–15% Linear Gradient | 345-0027 | 345-0028 | 345-0029 | 345-0030† | 345-0103 |
| 4–20% Linear Gradient | 345-0032 | 345-0033 | 345-0034 | 345-0035 | 345-0104 |
| 8–16% Linear Gradient | 345-0037 | 345-0038 | 345-0039 | 345-0040† | 345-0105 |
| 10–20% Linear Gradient | 345-0042 | 345-0043 | 345-0044 | 345-0045† | 345-0107 |
| 10.5–14% Linear Gradient | 345-9949 | 345-9950 | 345-9951 | — | 345-0106 |
| Criterion Stain-Free Gels | | | | | |
| 10% Tris-HCl Gel | 345-1012 | 345-1018 | — | — | — |
| 4–20% Tris-HCL Gel | 345-0412 | 345-0418 | 345-0426 | — | — |
| 8–16% Tris-HCL Gel | 345-8162 | — | 345-8166 | — | 345-8161 |
| Criterion Tris-Tricine Gels | | | | | |
| 16.5% Tris-Tricine | 345-0063 | 345-0064 | 345-0065† | 345-0066† | — |
| 10–20% Tris-Tricine | 345-0067 | 345-0068 | 345-0069 | — | — |
| Criterion IEF Gels | | | | | |
| pH 3–10 | 345-0071† | 345-0072† | 345-0073† | — | — |
| pH 5–8 | — | 345-0076† | — | — | — |
| Criterion Zymogram Gels | | | | | |
| 10% Zymogram Gel with Gelatin | 345-0079† | 345-0080† | 345-0081† | — | — |
| 12.5% Zymogram Gel with Casein | 345-0082† | 345-0083† | 345-0084† | — | — |

* Multichannel pipet compatible.
** Includes reference well(s).
*** Purchase of this product is accompanied by a limited license under U.S. Patent Numbers 6,143,154; 6,096,182; 6,059,948; 5,578,180; 5,922,185; 6,162,338; and 6,783,651 and corresponding foreign patents.
† Please allow up to 2 weeks for delivery.

| Catalog # | Description |
|---|--|
| Gel Casting Accessories See catalog or www.bio-rad.com for a complete listing of accessories, including available empty gel cassettes and glass plates, spacers, combs, etc. | |
| 165-5131 | AnyGel Stand, 6-row , holds 6 PROTEAN® gels, 12 Criterion gels, or 18 Ready Gel® mini gels |
| 165-4131 | AnyGel Stand, single-row , holds 1 PROTEAN gel, 2 Criterion gels, or 3 Ready Gel mini gels |
| 165-4122 | Model 485 Gradient Former and Mini-PROTEAN 3 Multi-Casting Chamber , includes 165-4120 and 165-4110 |
| 165-4123 | Model 495 Gradient Former and PROTEAN Plus Multi-Casting Chamber , includes 165-4121 and 165-4160 |
| Total Protein Gel Stains | |
| 161-0803 | QC Colloidal Coomassie Solution Kit , 1 L, ready-to-use, non-hazardous colloidal Coomassie G-250 stain for protein polyacrylamide gels |
| 161-0786 | Bio-Safe™ Coomassie Stain , 1 L |
| 161-0787 | Bio-Safe Coomassie Stain , 5 L |
| 161-0435 | Coomassie Brilliant Blue R-250 Staining Solutions Kit , includes 1 L Coomassie Brilliant Blue R-250 staining solution, 2 x 1 L Coomassie Brilliant Blue R-250 destaining solution |
| 161-0436 | Coomassie Brilliant Blue R-250 Staining Solution , 1 L |
| 161-0438 | Coomassie Brilliant Blue R-250 Destaining Solution , 1 L |
| 161-0400 | Coomassie Brilliant Blue R-250 , 10 g |
| 161-0406 | Coomassie Brilliant Blue G-250 , 10 g |
| 161-0443 | Silver Stain Kit , includes oxidizer concentrate, silver reagent concentrate, silver stain developer, stains 20 full size or 48 mini gels |
| 161-0449 | Silver Stain Plus™ Kit , includes fixative enhancer concentrate, silver complex solution, reduction moderator solution, image development reagent, development accelerator reagent, stains 13 full size or 40 mini gels |
| 161-0496 | Oriole™ Fluorescent Gel Stain , 1x solution, 1 L |
| 161-0492 | Flamingo™ Fluorescent Gel Stain , 10x solution, 500 ml |
| 170-3125 | SYPRO Ruby Protein Gel Stain , 1x solution, 1 L |
| 161-0440 | Zinc Stain and Destain Kit , includes 125 ml of 10x zinc stain solution A, 125 ml of 10x zinc stain solution B, 125 ml of 10x zinc destain solution |
| 161-0470 | Copper Stain and Destain Kit , includes 125 ml of 10x copper stain, 125 ml of 10x copper destain solution |
| High-Throughput Stainers | |
| 165-3400 | Dodeca™ Stainer, large , 100–240 V, includes 13 trays (12 clear, 1 white), 12 tray attachments, shaking rack, solution tank, lid with shaker motor, shaker control unit, gel clip |
| 165-3401 | Dodeca Stainer, small , 100–240 V, includes 13 trays (12 clear, 1 white), 12 Criterion tray attachments, shaking rack, solution tank, lid with shaker motor, shaker control unit, gel clip |

| Catalog # | Description |
|---|---|
| Imaging Systems | |
| 170-7991 | GS-900 Calibrated Densitometry System , gel densitometry system, PC compatible, scanner, cables, Image Lab software, optional 21 CFR Part 11 and Instrument Qualification/ Operations Qualification |
| 170-8195 | Gel Doc™ XR+ System with Image Lab Software , PC or Mac, includes darkroom, UV transilluminator, epi-white illumination, camera, cables, Image Lab software |
| 170-8270 | Gel Doc EZ System with Image Lab Software , PC or Mac, includes darkroom, camera, cables, Image Lab software; samples trays (#170-8271, 170-8272, 170-8273, or 170-8274) are sold separately; sample trays are required to use the system |
| 170-8265 | ChemiDoc™ XRS+ System with Image Lab Software , PC or Mac, includes darkroom, UV transilluminator, epi-white illumination, camera, power supply, cables, Image Lab™ software |
| 170-8280 | ChemiDoc MP System with Image Lab Software , PC or Mac, includes darkroom, UV transilluminaator, epi-white illumination, camera, power supply, cables, ImageLab™ software |
| 170-9460 | PharosFX™ Plus System , PC or Mac, 110–240 V, includes Quantity One® software, sample tray set, fluorescence filters (170-7866, 170-7896) and phosphor imaging filters, USB2 cable |
| 170-9450 | PharosFX System , PC or Mac, 110–240 V, includes Quantity One software, sample tray set, fluorescence filters (170-7866, 170-7896), USB2 cable |
| 170-9400 | Personal Molecular Imager™ (PMI) System , PC or Mac, 110/240 V, includes Quantity One software, sample tray set, USB2 cable |
| Analysis Software | |
| 170-9690 | Image Lab Software |
| 170-9600 | Quantity One 1-D Analysis Software , PC or Mac |
| 170-9630 | PDQuest Advanced 2-D Analysis Software |
| Gel Drying and Electroelution Supplies | |
| 165-1771 | GelAir™ Drying System , 115 V, 60 Hz, includes 165-1777, 2 drying frames, 16 clamps, assembly table, 50 precut sheets of cellophane support, gel drying solution |
| 165-1777 | GelAir Dryer , 115 V, gel drying oven only |
| 165-1251 | Whole Gel Eluter with Harvesting Box , includes lid, electrodes, elution chamber core, base, roller, ruler, template, 75 pieces of lower filter paper, 50 pieces of upper filter paper, 50 sealing strips, 25 pieces of cellophane, application note; requires a vacuum source pulling 5" Hg for the harvesting box |
| 165-1256 | Mini Whole Gel Eluter with Harvesting Box , includes lid, electrodes, elution chamber core, base, roller, ruler, template, 75 pieces of lower filter paper, 50 pieces of upper filter paper, 50 sealing tabs, 25 pieces of cellophane, application note; requires a vacuum source pulling 5" Hg for the harvesting box |
| 165-2976 | Model 422 Electro-Eluter , includes electro-eluter module, membrane caps (MW cutoff 12,000–15,000), glass tubes, frits, silicone adaptors, grommets and stoppers, buffer tank, lid with power cables |

Purchase of Criterion XT Bis-Tris gels, XT MOPS running buffer, XT MES running buffer, XT MOPS buffer kit, and XT MES buffer kit is accompanied by a limited license under U.S. Patent Numbers 6,143,154; 6,096,182; 6,059,948; 5,578,180; 5,922,185; 6,162,338; and 6,783,651, and corresponding foreign patents.



LabChip and the LabChip logo are trademarks of Caliper Life Sciences, Inc. Bio-Rad Laboratories, Inc. is licensed by Caliper Life Sciences, Inc. to sell products using the LabChip technology for research use only. These products are licensed under U.S. Patent Numbers 5,863,753; 5,658,751; 5,436,134; and 5,582,977, and pending patent applications, and related foreign patents, for internal research and development use only in detecting, quantitating, and sizing macromolecules, in combination with microfluidics, where internal research and development use expressly excludes the use of this product for providing medical, diagnostic, or any other testing, analysis, or screening services, or providing clinical information or clinical analysis, in any event in return for compensation by an unrelated party.

Precision Plus Protein standards are sold under license from Life Technologies Corporation, Carlsbad, CA, for use only by the buyer of the product. The buyer is not authorized to sell or resell this product or its components.

StrepTactin is covered by German patent application P 19641876.3. Bio-Rad Laboratories, Inc. is licensed by Institut für Bioanalytik GmbH to sell these products for research use only.

Bio-Rad Laboratories, Inc. is licensed by Invitrogen Corporation to sell SYPRO products for research use only under U.S. Patent Number 5,616,502.

Coomassie is a trademark of BASF Aktiengesellschaft. Cy is a trademark of GE Healthcare. NuPage, Pro-Q, and SYPRO are trademarks of Invitrogen Corporation. Strep-tag is a trademark of Institut für Bioanalytik GmbH. Triton is a trademark of Dow Chemical Corporation. Tygon is a trademark of Norton Company.



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Web site www.bio-rad.com **USA** 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 01 877 89 01 **Belgium** 09 385 55 11 **Brazil** 55 11 5044 5699
Canada 905 364 3435 **China** 86 21 6169 8500 **Czech Republic** 420 241 430 532 **Denmark** 44 52 10 00 **Finland** 09 804 22 00
France 01 47 95 69 65 **Germany** 089 31 884 0 **Greece** 30 210 9532 220 **Hong Kong** 852 2789 3300 **Hungary** 36 1 459 6100 **India** 91 124 4029300
Israel 03 963 6050 **Italy** 39 02 216091 **Japan** 03 6361 7000 **Korea** 82 2 3473 4460 **Mexico** 52 555 488 7670 **The Netherlands** 0318 540666
New Zealand 64 9 415 2280 **Norway** 23 38 41 30 **Poland** 48 22 331 99 99 **Portugal** 351 21 472 7700 **Russia** 7 495 721 14 04
Singapore 65 6415 3188 **South Africa** 27 861 246 723 **Spain** 34 91 590 5200 **Sweden** 08 555 12700 **Switzerland** 026674 55 05
Taiwan 886 2 2578 7189 **Thailand** 800 88 22 88 **United Kingdom** 020 8328 2000