

Mini Prep Cell

Instruction Manual

Catalog Number
170-2908



Table of Contents

| | Page |
|--|-----------|
| Section 1 General Information | 1 |
| 1.1 Introduction | 1 |
| 1.2 Accessory Equipment..... | 1 |
| 1.3 Specifications | 2 |
| 1.4 Chemical Compatibility | 2 |
| 1.5 Safety | 2 |
| Section 2 Description of Major Components | 3 |
| 2.1 Mini Prep Cell Components..... | 4 |
| Section 3 Assembly and Operation of the Mini Prep Cell | 4 |
| 3.1 Casting a Gel | 4 |
| 3.2 Preparing the Frits and Dialysis Membrane | 4 |
| 3.3 Assembling and Purging the Elution Chamber | 6 |
| 3.4 Assembly of Upper and Lower Buffer Chambers..... | 7 |
| 3.5 Filling the Buffer Reservoirs..... | 8 |
| 3.6 Loading the Sample..... | 8 |
| 3.7 Cooling the Gel | 8 |
| 3.8 Elution Rate | 8 |
| 3.9 Collection and Analysis | 8 |
| Section 4 Optimizing Running Conditions for SDS-PAGE | 9 |
| 4.1 Gel Pore Size | 9 |
| 4.2 Optimization Procedures..... | 10 |
| 4.3 Gel Length..... | 11 |
| 4.4 Running Conditions | 12 |
| 4.5 Elution Times for Proteins | 12 |
| Section 5 Disassembly and Cleaning | 13 |
| Section 6 Troubleshooting Guide | 14 |
| Section 7 Reagent Preparation for SDS-PAGE | 15 |
| 7.1 Electrophoresis Buffers and Acrylamide Stock Solutions for SDS-PAGE..... | 15 |
| 7.2 Separating Gel Buffer Stock | 15 |
| 7.3 Stacking Gel Buffer Stock | 15 |
| 7.4 Sample Buffer Stock | 15 |
| 7.5 Electrophoresis Running Buffer Stock | 15 |
| 7.6 Acrylamide Stock Solution Preparation | 16 |
| Section 8 Gel Preparation for Analytical and Preparative PAGE | 17 |
| 8.1 Gel Preparation Table for Analytical and Preparative SDS-PAGE..... | 17 |
| 8.2 Analytical Separating Gel Calculation | 18 |
| 8.3 Analytical Stacking Gel Calculation..... | 19 |
| 8.4 Preparative Stacking Gel and Resolving Gel Calculation..... | 19 |
| Section 9 Preparative Native-PAGE | 20 |
| 9.1 Introduction | 20 |
| 9.2 Native-PAGE Systems | 20 |
| 9.3 Discontinuous Native-PAGE | 23 |
| 9.4 Continuous Native-PAGE..... | 26 |
| 9.5 References | 29 |
| Section 10 Product Information | 30 |

Model _____

Catalog No. _____

Date of Delivery _____

Warranty Period _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

Warranty

Bio-Rad Laboratories' Mini Prep Cell is warranted against defects in materials and workmanship for 1 year from date of purchase. If any defects occur in the instrument during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts free. The following defects, however, are specifically excluded:

1. Damage caused by improper operation, accident, or misuse.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of fittings or other parts supplied by anyone other than Bio-Rad Laboratories.

Note: This warranty does not apply to platinum wire electrodes.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument.

Section 1

General Information

1.1 Introduction

The Mini Prep Cell* purifies specific proteins or nucleic acids from complex mixtures by continuous-elution electrophoresis. Conventional gel electrophoresis buffer systems and media are used with the Mini Prep Cell.

During a run, samples are electrophoresed through a cylindrical gel. As molecules migrate through the gel matrix, they separate into bands. Individual bands migrate off the bottom of the gel where they pass directly into the patented elution chamber for collection.

The elution chamber consists of a thin polyethylene frit. A dialysis membrane, directly underneath the elution frit, traps proteins within the chamber. Elution buffer enters the chamber around the perimeter. The unique design of the chamber results in an even flow of buffer into the elution frit. Buffer is drawn radially inward toward an elution tube. Purified molecules are drawn down and out through the elution tube by a peristaltic pump. The peristaltic pump drives separated proteins to a fraction collector (Bio-Rad's Econo System).

Simple procedures are provided for determining optimal running conditions for most purifications. It is recommended that these procedures be performed for each new sample to be purified before proceeding to a preparative run with the Mini Prep Cell.

* U.S. patent number 4,877,510

1.2 Accessory Equipment

Required:

Power supply - 300 volt

Peristaltic pump - 100 μ l/minute

Fraction collector*

*The use of a reliable peristaltic pump and fraction collector is essential for the isolation of the desired component of the sample. To simplify set-up and operation of accessory equipment, we recommend use of Econo System low pressure chromatography components, including peristaltic pump and fraction collector.

1.3 Specifications

Construction

| | |
|-----------------------------|-------------------------------|
| Upper buffer chamber | acrylic |
| Lower buffer chamber | acrylic |
| Electrodes | platinum, 0.010 inch diameter |
| Lid | acrylic |
| Gel tube assembly | glass/silicone rubber |
| Elution chamber base | acrylic |
| Elution frit | polyethylene |
| Support frit | polyethylene |
| Elution tubing | PTFE - 0.06" ID |
| Harvest ring | molded polyphenylsulfide |
| Casting stand | acrylic |

Other Specifications

| | |
|--|-----------------------------|
| Shipping weight | 3 lb |
| Overall size | 5. in diameter x 10 in high |
| Voltage limit | 500 volts |
| Current limit | 10 milliamperes |
| Power limit | 5 watts |
| Elution buffer flow rate | 100 µl/minute |
| Upper electrophoresis buffer volume | 100 ml |
| Elution buffer chamber volume | 100 ml |
| Lower electrophoresis buffer volume | 400 ml |

1.4 Chemical Compatibility

The Mini Prep Cell is not compatible with chlorinated hydrocarbons (e.g. chloroform), aromatic hydrocarbons (e.g. toluene, benzene), or acetone. Their use will void all warranties.

1.5 Safety

Power to the Mini Prep Cell is to be supplied by an external DC power supply. This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground. The recommended power supply for this instrument is the PowerPac 3000 computer controlled power supply. The maximum specified operating parameters for the Mini Prep Cell are:

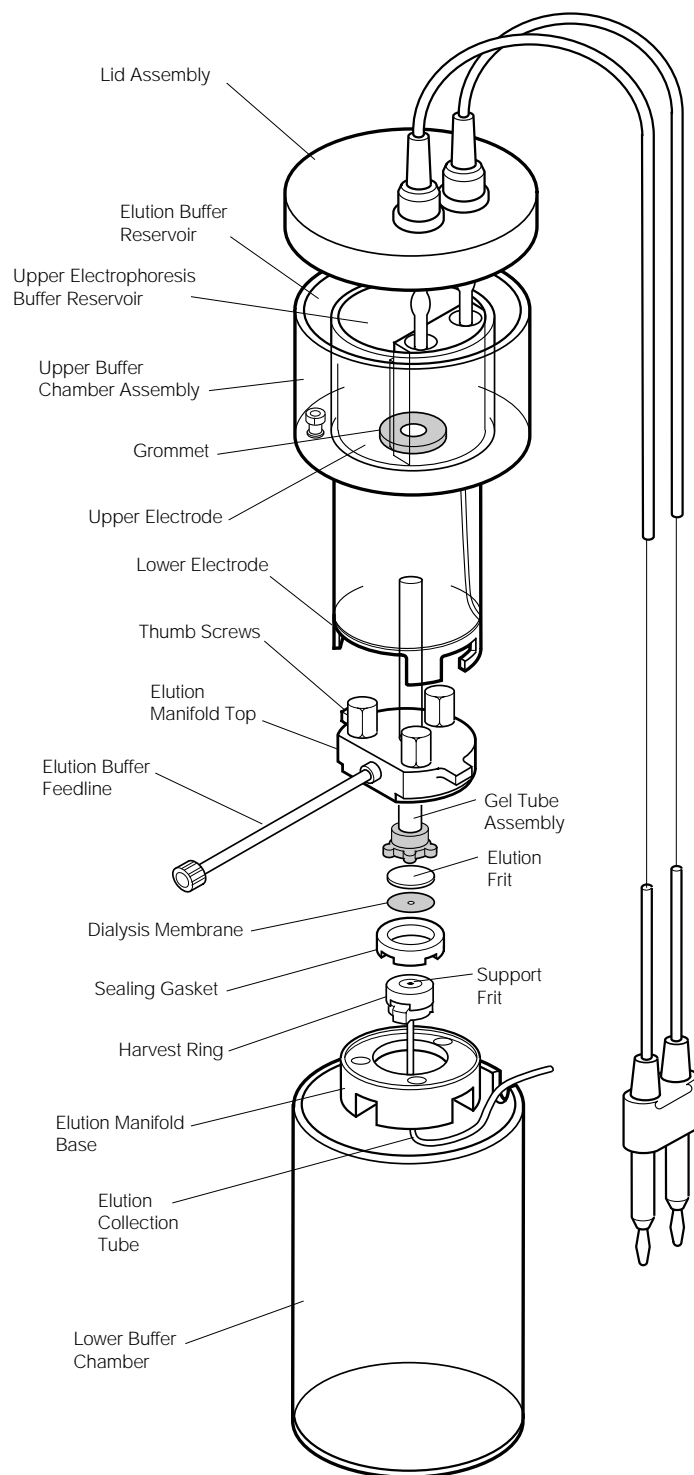
| | |
|----------------|-------------------------------|
| 500 VDC | maximum operating voltage |
| 10 mA | maximum operating current |
| 5 W | maximum operating power limit |

Current to the Mini Prep Cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current flow to the cell is broken when the lid assembly is removed. Do not attempt to circumvent this safety interlock, and always turn the power supply off when working with the cell.

The recommended power supplies are ground isolated by design to minimize the potential shock hazard. However, working around high voltage equipment in a laboratory environment is potentially dangerous. It is the user's responsibility to always exercise care in setting up and running electrophoresis instruments. If a liquid leak occurs, always turn off the power supply before correcting the problem.

During operation, do not expose the cell to ambient temperatures above 50 °C.

Section 2 Description and Assembly of Components



Mini Prep Cell - Exploded View.

2.1 Mini Prep Cell Components

Upper and Lower Buffer Chamber

The lower electrophoresis buffer chamber forms a stable base for the unit. The upper buffer chamber assembly consists of an upper electrophoresis buffer reservoir and an elution buffer reservoir. The upper buffer chamber assembly also houses the upper and lower electrodes.

Gel Tube Assembly

The inner diameter of the glass gel tube is 7 mm. A rubber gasket is bonded to the lower end. Its purpose is to seal the gel tube in the elution chamber. Two 13 cm gel tubes are provided with the Mini Prep Cell.

Elution Manifold

The elution manifold consists of several parts: the elution manifold top holds the gel tube and contains three thumb screws. The elution manifold base contains a grey sealing gasket and holds the elution buffer collection assembly.

Elution Buffer Collection Assembly

The elution buffer collection assembly controls the flow of elution buffer so that eluted molecules do not remix and are harvested with minimal dilution. The collection assembly consists of an elution frit, a precut dialysis membrane, the molded harvest ring, a support frit and the elution collection tube.

The pre-cut dialysis membranes provided with the Mini Prep Cell have molecular weight cut offs of 6,000 and 3,500 daltons. Small molecules such as the electrophoresis tracking dye (bromophenol blue, ~691 daltons, peptides or oligonucleotides, should not pass through the dialysis membrane. This is because the horizontal flow of elution buffer through the elution frit overcomes the force of electrophoresis in the vertical direction.

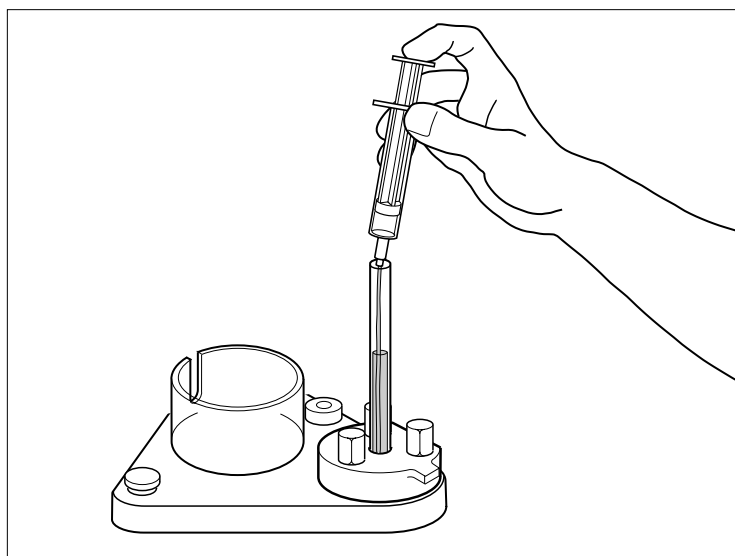
Casting Stand

Gels are cast with the gel tube mounted on the casting stand. The casting stand insures that gels have flat lower surfaces. Leveling feet allow for casting of gels with flat upper surfaces. The casting stand also serves to support the gel tube and elution chamber during assembly of the upper buffer chamber.

Section 3 Assembly and Operation

3.1 Casting a Gel

1. Place the gel tube assembly and the elution chamber top on the casting stand, aligning the three thumb screws with the holes in the casting stand. Secure the gel tube assembly with the three screws; hand tightening is sufficient. Level the casting stand with the aid of the leveling bubble by adjusting the leveling legs.



2. Prepare the acrylamide monomer solution. Refer to Section 4 for selecting the appropriate acrylamide gel concentration for a given application. Sections 8 and 9 describe preparative gel formulations for SDS-PAGE and native PAGE, respectively.
3. Using a small syringe with a PTFE tube affixed or disposable pipette, slowly inject the degassed monomer mixture into the gel tube filling from the bottom. To avoid trapping air bubbles in the gel, gently tap the casting stand (with the gel tube assembly mounted to it) against the bench top. This will help to dislodge trapped air bubbles. Visually inspect the bottom of the gel for bubbles immediately after pouring the gel solution into the tube.

Carefully overlay the gel with water-saturated 2-butanol or tert-amyl alcohol using the narrow PTFE tube with a syringe (provided with the unit). Allow the resolving gel to stand overnight for complete polymerization (catalyst concentration in resolving gel is 0.025% APS/0.025% TEMED). After 1-2 hours polymerization, replace the alcohol overlay with gel buffer. In the case of SDS-PAGE and Ornstein-Davis non-denaturing gels, this should be 0.375M Tris/Cl, pH 8.8 buffer.

4. Very carefully decant or aspirate the buffer overlay. Cast the stacking gel, approximately twice the sample volume, on top of the resolving gel. Overlay the stacking gel monomer with water-saturated 2-butanol or tert-amyl alcohol. Allow the stacking gel to polymerize for 1-2 hours.

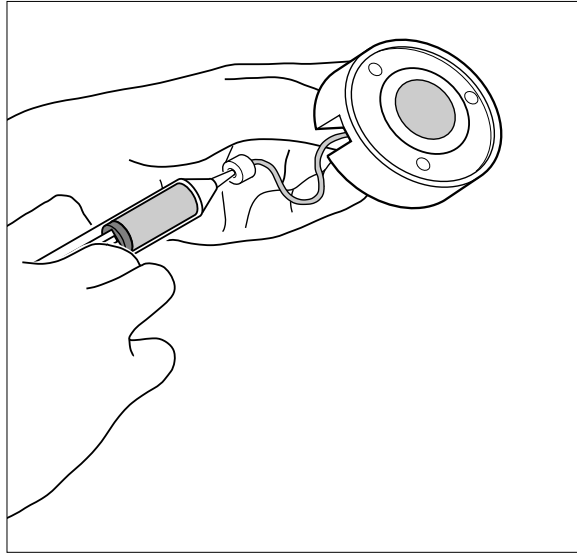
3.2 Preparing the Frits and Dialysis Membrane

Place the molded harvest ring of the elution buffer collection assembly (containing the support frit), along with the elution frit and dialysis membrane in electrophoresis buffer. The frits and dialysis membrane must be completely wetted prior to use. To insure removal of entrapped air in the pores of the frits, place the container in which the frits are soaking in a vacuum chamber for approximately 10 minutes. Alternatively, the frits can be soaked in buffer overnight to completely wet them. To maintain the wetting of the frits, store them in buffer or water. Incomplete wetting results in high voltage due to an increase in electrical resistance.

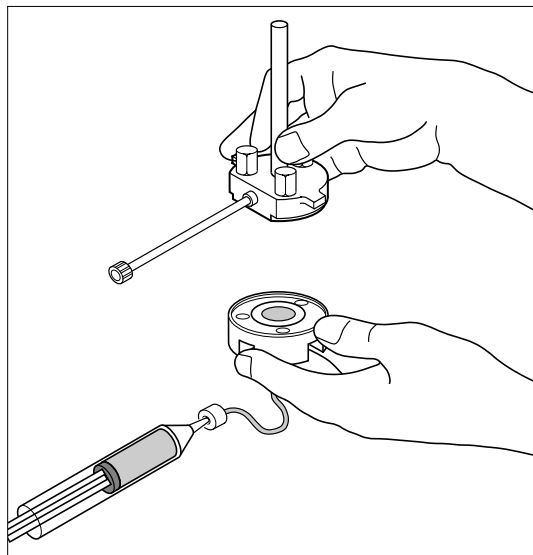
The dialysis membrane must be soaked in buffer before use and stored in buffer or water between uses. If the membrane becomes dry between runs, discard it. A properly stored dialysis membrane can be used at least for 5-6 runs. Prior to each run, inspect the membrane carefully. Discard it if any holes or tears are detected.

3.3 Assemble and Purge the Elution Chamber

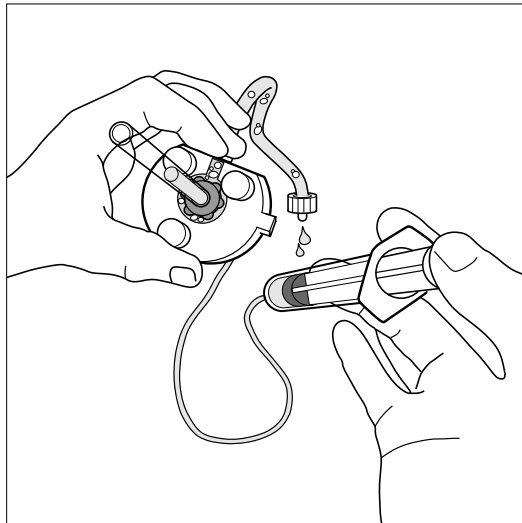
After soaking the support frit, elution frit, and dialysis membrane in electrophoresis buffer, place the molded harvest ring of the elution buffer collection assembly into the lower portion of the elution chamber. Then press in the gray sealing gasket around the harvest ring to hold the assembly in place



To remove air bubbles and fill the elution chamber with elution buffer, fill a syringe with electrophoresis buffer and connect it to the Luer fitting of the 1 mm PTFE elution collection tubing exiting the base of the elution manifold. Gently push elution buffer through the elution tubing (do not pull) until buffer fills the space within the gray sealing gasket. Then, place the dialysis membrane on top of the support frit and place the elution frit on top of the dialysis membrane.

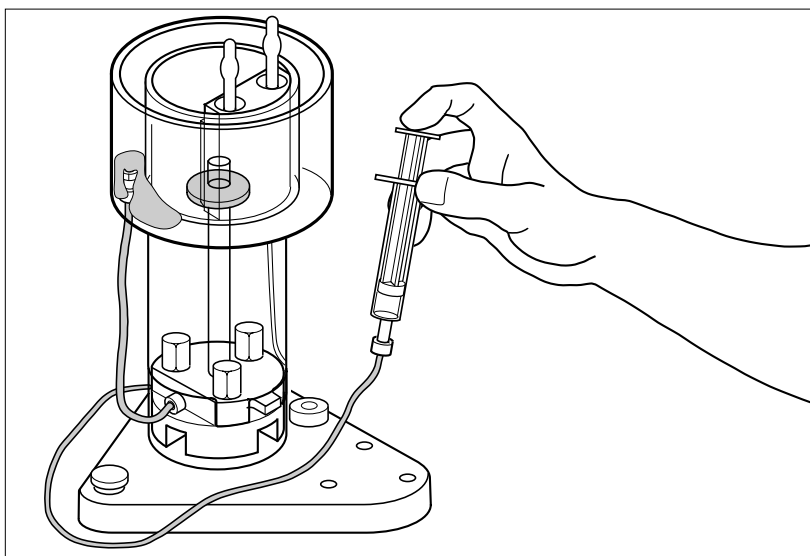


Remove the gel tube from the casting stand and place the assembly onto the lower portion of the elution manifold. Align the three screws of the elution manifold top with the threaded holes in the lower portion and hand tighten them.



Continue purging the elution system until elution buffer flows out through the elution buffer feedline. Visually inspect the elution chamber for air bubbles. If bubbles persist, tap the elution chamber against the lab bench to free the bubbles then gently push elution buffer through the system until the bubbles flow out through the elution buffer feedline.

3.4 Assembly of Upper and Lower Buffer Chambers



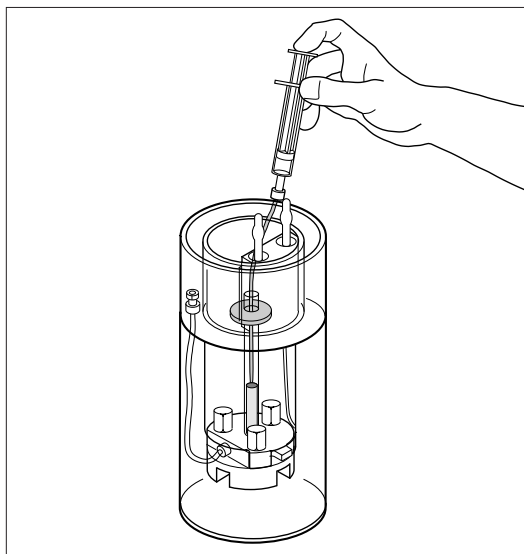
Place the assembled elution chamber/gel tube assembly into the cylindrical holding station on the casting stand. Allow the elution buffer collection tube to pass out through the slot in the holding station. Slide the top of the gel tube into the grommet in the center of the upper buffer chamber. Align the two wings protruding from the elution manifold with the slots in the lower electrode housing. Twist and lock the two components in place. Connect the elution buffer feed line (female Luer fitting) to the male Luer fitting attached to the elution buffer reservoir. Gently push elution buffer through the elution collection tube to prime the elution system. Priming is complete when buffer flows into the elution buffer reservoir.

3.5 Fill the Buffer Reservoirs

Fill the buffer reservoirs with appropriate electrophoresis buffer. The upper buffer reservoirs each hold 100 ml. Add 400 ml electrophoresis buffer to the lower buffer chamber. The level of buffer should just reach the bottom edge of the gel tube. During a run, the gel tube should not be submerged in lower electrophoresis buffer. Place the upper buffer chamber/gel tube assembly into the lower chamber to check that this is so. Sections 8 and 9 describe electrophoresis buffers for SDS-PAGE and native-PAGE, respectively.

3.6 Load the Sample

Carefully load the sample on the surface of the stacking gel with the 1 ml syringe. Layer the sample under the electrophoresis buffer. Make sure the stacking gel is not punctured with the PTFE loading tube. Once the sample is loaded, place the lid on the cell and attach the cables to the power supply. Set the power supply to the appropriate settings and begin electrophoresis.



3.7 Cooling the Gel

For separation of native proteins where colder temperatures are desirable, the Mini Prep Cell can be run in a cold room to keep fractions cold once they have been collected.

3.8 Elution Rate

We recommend an elution buffer flow rate of 75-100 $\mu\text{l}/\text{min}$. Fractions collected for 2.5 minutes (~200-250 μl) usually maintain sufficient separation of eluted proteins. Fraction collection should begin after the ion/dye front has eluted. For preparative SDS-PAGE, table 4.5 shows the approximate elution times of purified proteins for gels run with the optimum %T and proper power conditions. Refer to Section 9 for native PAGE.

3.9 Collection and Analysis

Electrophoretic analysis of individual fractions is recommended for determining the distribution of proteins and the level of contamination in each fraction. To determine which fractions contain the protein of interest, individual fractions should be analyzed by SDS-PAGE and silver stained. It is recommended that an analysis be performed by running every fifth or tenth fraction past the ion front (*i.e.*, fractions collected every 15 to 30 minutes) in mini gels.

When the region with the protein of interest is identified, every fraction within that region should be analyzed to determine purity obtained. Fractions containing the purified protein can be pooled and concentrated as required for further analysis.

Section 4

Optimizing Running Conditions for Preparative SDS-PAGE (Laemli)

The Mini Prep Cell for separates a single component from its nearest contaminant. The conditions required to achieve optimum resolution may be different than those of analytical electrophoresis. In analytical SDS-PAGE, optimum resolution is needed over a wide molecular weight range, whereas in preparative SDS-PAGE only the protein of interest needs to be separated from its nearest contaminant.

The three variables to be considered for optimum resolution in preparative SDS-PAGE are:

- Gel pore size
- Gel optimization procedure
- Gel length

4.1 Gel Pore Size (Determining Optimum %T)

Since each protein purification is unique, it is important to first optimize the running conditions for each application on an analytical level prior to preparative fractionation. In general, the best purification will be achieved if the sample is at least partially purified prior to a preparative run. Any optimization procedure should be carried out using the same protein sample as will be applied to the Mini Prep Cell.

The most important parameter in preparative SDS-PAGE is the pore size of the gel. The gel pore size is a function of the acrylamide monomer concentration used (%T) to cast a gel. The monomer concentration which best resolves two protein bands will vary depending on the molecular weights of the proteins of interest. Changing the gel composition from the optimal concentration, by increasing or decreasing the monomer concentration, will ultimately decrease resolution.

By convention, polyacrylamide gels are characterized by the figures (%T/%C), where %T is the weight percentage of total monomer including crosslinker (in g/100 ml), and %C is the proportion of crosslinker as a percentage of total monomer. For both the analytical gels and the preparative gels use 2.67% N, N'-methylene-bis-acrylamide crosslinker. Premixed acrylamide:bis, in the ratio 37.5:1, can also be used (See section 7). The total monomer concentration for optimal resolution is referred to as optimal %T.

The pore size providing the optimum resolution for most proteins is that which results in a relative mobility (R_f) value between 0.55-0.6. This is an empirical finding, the reasons for which are not entirely clear.

R_f values for specific proteins are obtained from the mini-gels that were run to optimize conditions for the Mini Prep Cell. To calculate R_f values for specific proteins use this formula:

$$R_f = \frac{\text{Distance migrated by the protein of interest}}{\text{Distance migrated by the ion front}}$$

The R_f value obtained from a mini gel can be used to estimate when a protein will elute from the Mini Prep Cell run with the same concentration of acrylamide. For those samples in which the molecular weight difference between the protein of interest and its nearest contaminant is $\geq 10\%$ the optimum %T can be selected from Figure 4.1. For those samples in which the molecular weight difference between the protein of interest and its nearest contaminant is less than 10%, consult Section 4.3.

4.2 Optimization Procedures

Simplified Optimization Procedure

When the difference in the molecular weights of the protein of interest and its nearest contaminant is 10% or greater, select the monomer concentration (optimum %T) which corresponds to the size of the protein of interest from the plot below. In most cases the %T so obtained will provide adequate resolution for the purified protein.

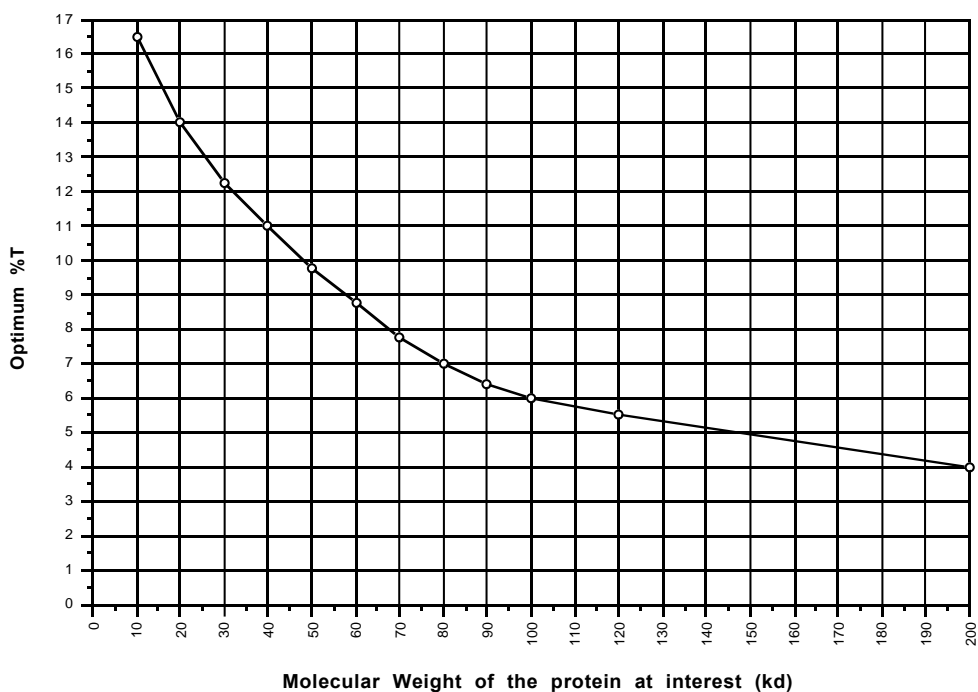


Fig. 4.1. Mini Prep Cell SDS-PAGE gel optimization curve. In cases where the difference in molecular weight between the protein of interest and its nearest contaminant is 10% or greater, the appropriate acrylamide concentration to purify the protein of interest from its nearest contaminant can be determined using this standard curve.

Detailed Optimization Procedure

For samples where the molecular weight of the molecule of interest differs from its nearest contaminant by less than 10%, we recommend the following procedure. Preliminary optimization is done using mini-slab gels, thus eliminating lengthy and wasteful trial runs on the Mini Prep Cell.

The detailed optimization procedure involves running a series of analytical mini-gels covering a range of %T (in 1-2%T increments). Table 4.2 gives recommended ranges of monomer concentrations for various protein molecular weights. Use Figure 4.1 to obtain a midpoint for the range of gels to cast.

Table 4.2 Recommended Monomer Concentrations

| Size Range | %T Range |
|--------------|----------|
| 15 - 30 kd | 10 - 16% |
| 30 - 50 kd | 9 - 12% |
| 50 - 70 kd | 7 - 10% |
| 70 - 100 kd | 5 - 9% |
| 100 - 200 kd | 4 - 8% |

The procedure is as follows:

1. Cast several polyacrylamide mini-slab gels in the range suggested in Table 4.2 (and Figure 4.1).
2. Load a sufficient amount of protein for detection by silver staining (~100 ng/lane). Load at least one lane with 10 μ l prestained high or low molecular weight standards and one lane with SDS PAGE silver stain standards (optional).
3. Choose one of the prestained molecular weight standards which migrates closest to the molecular weight of the proteins of interest to monitor the gel run. Start electrophoresis and continue until the prestained marker protein has either run off the end of the gel or, in the case where the proteins of interest are smaller than the marker, until the standard protein has reached a designated location near the end of the gel.
4. When electrophoresis is complete, silver stain the gels and dry them.
5. Measure the distance between the band of interest (molecule to be purified) and its nearest contaminant in each gel. (Net distance is measured; *i.e.*, measure the distance between the bottom of the upper band and the top of the lower band.)
6. Plot the distance between bands versus %T and determine optimal %T from the cusp (or breakpoint) of the graph. This is the optimal %T for use in the preparative gel tube. Resolution decreases at either side of optimal %T.

4.3 Gel Length Determination for Preparative SDS-PAGE

Gel length is used to increase the separation between proteins. However, longer gels are accompanied by an increase in band width. The minimal gel length depends on the difference in molecular weight between the protein of interest and its nearest contaminant. The size difference between these two proteins (Δ MW) is inversely related to the recommended gel length. Small size differences require longer gels to produce the best separation. The sample load, *i.e.* the amount of the protein of interest and its nearest contaminant, also affects resolution. Resolution can be improved by either decreasing the sample load or increasing

the gel length. Refer to Table 4.3 to select the gel length for optimizing resolution and recovery of the protein of interest.

The following are guidelines for optimizing resolution and recovery of the protein of interest with the least amount of dilution. The recommended protein loads in Table 4.3 refer to the amount of the protein of interest and its nearest contaminant, not the total protein load. The tables also provide the monomer volumes required for the recommended gel length.

Table 4.3

| Δ MW ^a | Protein Load ^b | Monomer Volume ^c | Gel Length |
|--------------------------|---------------------------|-----------------------------|------------|
| >15% | <100 μ g | 2 ml | 5 cm |
| >15% | 100-200 μ g | 2-4 ml | 5-10 cm |
| 10-15% | <75 μ g | 2 ml | 5 cm |
| 10-15% | 75-100 μ g | 2-4 ml | 5-10 cm |
| 2-10% | <50 μ g | 2 ml | 5 cm |
| 2-10% | 50-75 μ g | 2-4 ml | 5-10 cm |

- (a) Δ MW refers to the percentage difference in size between the protein of interest and its nearest contaminant.
- (b) Protein load refers to the combined amount of the protein of interest and its nearest contaminant only and is independent of the total protein load. Loads greater than the amounts recommended here may lead to corresponding loss of resolution.
- (c) The monomer volume recommended is based on using the optimum %T as established from analytical gels.

4.4 Running Conditions

| | | | | |
|---------------------|------------------|-----------|------------|---------|
| SDS-PAGE: | Constant Power | 1 W | ~175-250 V | ~3-6 mA |
| | Constant Voltage | 200 V | ~1 W | ~3-6 mA |
| Native PAGE: | Constant Power | 0.5 - 1 W | ~200-500 V | ~2-5 mA |

The running conditions for native PAGE are greatly dependent on the selected buffer system. See Section 9 for native PAGE.

4.5 Elution Times for Proteins

Approximate elution times for proteins run through preparative SDS-PAGE gels using 1 watt constant power are listed in Table 4.5. When SDS-PAGE is carried out at the optimum %T, the relative mobility (R_f) for the protein of interest will be about 0.55-0.6. This corresponds to a velocity of approximately half that of the ion front, regardless of the size of the proteins. Therefore, the elution time for preparative SDS-PAGE is affected by gel length and can be predicted. The elution times for both the ion/dye front and the purified protein are provided in Table 4.5

Table 4.5 Elution times at optimal %T. The elution times for proteins are approximate and will vary somewhat depending on the exact run conditions.

| Gel Length (cm) | Elution Time (hours) ($R_f \sim 0.55$) | Elution time for Ion Front ($R_f=1$) |
|----------------------------|--|--|
| 4 | 3.0 | 1.75 |
| 5 | 3.5 | 2 |
| 6 | 4.5 | 2.25 |
| 7 | 5.25 | 2.75 |
| 8 | 5.75 | 3.0 |
| 9 | 6.5 | 3.5 |
| 10 | 7.25 | 3.75 |

Section 5 Disassembling and Cleaning

1. Turn off the power supply, disconnect the cables from the power supply, and remove the lid. Turn off the elution pump.
2. Remove the upper reservoir with the gel tube assembly from the lower buffer chamber and pour off the remaining buffer from both the elution and upper electrophoresis buffer reservoirs. Disconnect the elution buffer feedline. Disconnect the elution tube from the peristaltic pump. Remove the upper reservoir from the gel tube assembly.
3. Loosen the screws in the elution chamber cap and remove the gel tube assembly from the elution chamber. Rinse the support frits and the elution frit with deionized water and place them in a container of buffer or water. Inspect the dialysis membrane for tears. If the dialysis membrane is intact, rinse it in deionized water and place it in a container with buffer or water.
4. Hold the gel tube in one hand, carefully force the gel out of the tube with a spatula. Use the wire brush provided to remove residual polyacrylamide from the gel tube. Wash the gel tube with Bio-Rad Cleaning Concentrate (catalog number 161-0722). Do not scratch or otherwise abrade the interior surface of the glass tube. Check for chipping at the bottom edge of the gel tube.
5. Decant the buffer from the lower reservoir. Rinse the Mini Prep Cell components in deionized water and dry them in air.

Section 6

Troubleshooting Guide

| Problem | Cause | Solution |
|--|--|--|
| 1. Sample requires a long time to enter the gel. | a. High salt concentration in the sample. | a. Remove salts by dialysis, determine salting column, etc. |
| 2. Poor resolution | a. Sample overloaded. b. Incorrect %T. c. Incorrect gel length. | a. Decrease sample load. b. Refer to section 4 c. Check Table 4.2. |
| 3. No detectable proteins in collected fractions. | a. Proteins too dilute. b. Insufficient protein load. c. %T too high and proteins remain in gel or diffuse to undetectable level. d. %T too low, protein migrates with ion front. | a. Use silver stained SDS-PAGE gels to analyze individual Mini Prep Cell fractions. b. Increase total protein loaded. c. Decrease %T. See detailed optimization procedure in Section 4. d. Increase %T. See detailed optimization procedure in Section 4. |
| 4. Elution buffer floods down into lower buffer chamber. | a. Dialysis membrane in elution chamber missing or damaged. | a. Check to see if dialysis membrane is in place and not damaged. |
| 5. Air pockets between gel and gel tube. | a. Mechanical stress. b. It is normal for a gel to pull slightly away from the wall. c. Polymerization too fast. | a. Reduce handling of the gel during polymerization. b. Refractive phenomena often accentuate the appearance of these regions. They will not affect protein resolution. c. Check catalyst concentration. |
| 6. Running conditions outside recommended range. | a. Buffer concentration or pH are incorrect. | a. Make fresh buffer. |

Section 7 Reagent Preparation

7.1 Electrophoresis Buffers and Acrylamide Stock Solutions for SDS-PAGE

The Mini Prep Cell has three buffer reservoirs: one for the lower electrophoresis buffer (~400 ml), one for upper electrophoresis buffer (~100 milliliters) and one for the elution buffer (~100 milliliters). For SDS-PAGE all three reservoirs contain the same buffer (Laemmli buffer system). Premixed liquid acrylamide (concentrated solution) and Premixed electrophoresis buffers (10x) are available (see product information section). Section 8 describes preparation of polyacrylamide gels using the following gel buffer stock solutions.

7.2 Separating (resolving) Gel Buffer Stock

1.5 M Tris-HCl pH 8.8

Dissolve 27.2 grams Tris base in approximately 100 ml deionized water.

Adjust to pH 8.8 with HCl.

Make to 150 ml with deionized water and store at 4 °C.

7.3 Stacking Gel Buffer Stock

0.5 M Tris-HCl, pH 6.8

Dissolve 6 grams Tris base in approximately 60 ml deionized water.

Adjust to pH 6.8 with HCl.

Make to 100 ml with deionized water and store at 4 °C.

7.4 Sample Buffer (SDS-reducing buffer)

| | |
|---|---------------|
| Deionized water | 2.8 ml |
| 0.5 M Tris-HCl, pH 6.8 | 1.0 ml |
| Glycerol | 2.0 ml |
| 10% (w/v) SDS* | 1.6 ml |
| <u>0.5% bromophenol blue (optional)</u> | <u>0.2 ml</u> |
| total volume | 7.6 ml |

Add 50 µl beta-mercaptoethanol to 950 µl of sample buffer prior to use. Mix equal volumes of sample buffer and sample, and heat at 95 °C for 4 minutes. SDS reducing buffer is 0.06 M Tris-HCl, pH 6.8, 2% SDS, 5% beta-mercapto-ethanol, 25% glycerol (w/v) and 0.01% bromophenol blue.

*To make a 10% SDS solution: Dissolve 1.0 grams of SDS in deionized water with gentle stirring and bring to 10 ml with water.

7.5 10x Electrode (Running) Buffer, pH 8.3 (Makes 1 Liter)

| | |
|------------------|---------|
| Tris base | 30.3 g |
| Glycine | 144.0 g |
| SDS | 10.0 g |

Dissolve and adjust to 1,000 ml with deionized water. DO NOT adjust pH with acid or base. To make 1 liter of 1X electrophoresis buffer (0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3) for the Mini Prep Cell, dilute 100 ml of 10X stock with 900 ml deionized water.

7.6 30% Acrylamide Stock Solution

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

| | |
|-------------------------------------|---------|
| Acrylamide | 146.0 g |
| N’N’-bis-methylene-acrylamide (Bis) | 4.0 g |

Dissolve in about 350 ml deionized water then adjust to 500 ml with deionized water. Filter and store at 4 °C in the dark.

(Prewighed Acrylamide/Bis 37.5:1 mixture or 40% Acrylamide/Bis Stock solution can be substituted. Calculate the volume of a 40% stock solution required to make the desired gel in Section 8.2.)

Caution: Acrylamide monomer is a neurotoxin. Avoid breathing acrylamide dust, do not pipet acrylamide solutions by mouth, and wear gloves when handling acrylamide powder or solutions containing it. For disposal of unused acrylamide, add bis-acrylamide (if none is present), induce polymerization, and discard the solidified gel.

Section 8 Gel Preparation

8.1 Prepare SDS-PAGE Gels of Any %T

The following table can be used to prepare both analytical and preparative Tris-HCl acrylamide gels. Sections 8.2 - 8.4 provide detailed formulas for calculating specific gel types. Acrylamide/bis stock solution of 30% (37.5:1) are used. The amounts listed for the components in the table below are based on a total volume of 10 ml. When SDS is included in the sample buffer and the upper electrophoresis running buffer it can be left out of the gels during their preparation.

Table 8.1 Gel Preparation.

| %T (acrylamide monomer) | DDI H ₂ O (ml) | Gel buffer solution* (ml) | Acrylamide / bis solution 30% stock (37.5:1) (ml) |
|-------------------------------|------------------------------|---------------------------------|---|
| 4% | 6.15 | 2.50 | 1.33 |
| 5% | 5.80 | 2.50 | 1.67 |
| 6% | 5.55 | 2.50 | 2.00 |
| 7% | 5.15 | 2.50 | 2.33 |
| 8% | 4.80 | 2.50 | 2.67 |
| 9% | 4.47 | 2.50 | 3.00 |
| 10% | 4.17 | 2.50 | 3.33 |
| 11% | 3.80 | 2.50 | 3.67 |
| 12% | 3.47 | 2.50 | 4.00 |
| 13% | 3.15 | 2.50 | 4.33 |
| 14% | 2.80 | 2.50 | 4.67 |
| 15% | 2.47 | 2.50 | 5.00 |
| 16% | 2.15 | 2.50 | 5.33 |
| 17% | 1.80 | 2.50 | 5.67 |

*Resolving Gel buffer - 1.5 M Tris-HCl, pH 8.8

*Stacking Gel buffer - 0.5 M Tris-HCl, pH 6.8

| Catalysts | 10% APS* | TEMED* |
|-------------------------------------|----------|--------|
| Analytical Resolving Gel | 50 µl | 5 µl |
| Stacking gel | 50 µl | 10 µl |
| Preparative Resolving Gel | 25 µl | 2.5 µl |
| Stacking Gel | 50 µl | 10 µl |

NOTE: *Amounts are per 10 ml gel volume. Different amounts of catalyst are added for analytical and preparative gels. To make 10% APS, dissolve 100 mg in 1 ml of deionized water. TEMED is used neat.

8.2 Analytical Separating Gels – Calculating %T (0.375 M Tris, pH 8.8)

| Monomer Concentration (% T/2.67 % C) | %T = x(%) |
|--|--|
| Acrylamides / Bis - 30%T | c ml = 30% |
| or for [40%T Stock]* | [c ¹ ml = 40%] |
| Deionized water | d ml |
| 1.5 M Tris-HCl, pH 8.8 | 2.5 ml |
| 10% Ammonium Persulfate (fresh daily) | 50 μl |
| TEMED | 5 μl |
| TOTAL MONOMER | 10 ml (volume needed for two mini gels) |

Determine (c ml) and (d ml) for 10 ml of total monomer:

c ml: Calculate the volume of 30% (or 40%)

Acrylamide/Bis stock

required for 10 ml of the desired total
monomer concentration

with the following formula:

$$\text{For 30\% stock: } c \text{ ml} = (x\%)(10 \text{ ml})/(30\%) = (x)(0.333) \text{ ml}$$

c¹ ml: *[Similarly, for 40% stock solution: c¹ ml = (x%)(10ml)/40%
= (x)(0.25)ml]

d ml: Calculate the volume of water required at the
desired total monomer concentration with the
following formula:

$$d \text{ ml} = (10 \text{ ml} - 2.5 \text{ ml} - 50 \mu\text{l} - 5 \mu\text{l}) - c \text{ ml} = 7.445 \text{ ml} - c \text{ ml}$$

Important: One can prepare any desired volume of monomer solution by multiplying the 10 ml recipe by the appropriate multiplying factor.

8.3 Analytical Stacking Gel – 4%T (0.125 M Tris, pH 6.8)

| Monomer Concentration (% T/2.67 % C) | %T = x(%) |
|--|--|
| Acrylamide / Bis - 30% T | c ml = 30% |
| or for [40% T Stock]* | [c ¹ ml = 40%] |
| Deionized water | d ml |
| 1.5M Tris-HCl, pH 8.8 | 2.5 ml |
| 10% Ammonium Persulfate (fresh daily) | 50 µl |
| TEMED | 5 µl |
| TOTAL MONOMER | 10 ml (volume needed for two mini gels) |

8.4 Preparative SDS-PAGE Separating and Stacking Gels- Calculating %T

| Monomer Concentration (%T/2.67%C) | Separating gel %x = %T | Stacking gel 4% T |
|---|---------------------------|----------------------|
| Acrylamide / Bis 30%T/2.67%C Stock Solution | c ml | 1.3 ml |
| Deionized water | d ml | 6.2 ml |
| 1.5 M Tris-HCl pH 8.8 | 10.0 ml | |
| 0.5 M Tris-HCl pH 6.8 | | 2.5 ml |
| 10% Ammonium Persulfate | 100 µl | 50.0 µl |
| TEMED | 10 µl | 10.0 µl |
| TOTAL MONOMER | 40 ml | 10.0 ml |

c ml: Calculate the volume of Acrylamide/Bis stock required for the desired total monomer concentration with the following formula:

$$\text{For 30\% stock: } c \text{ ml} = (x\%)(40 \text{ ml})/\% \text{ acrylamide stock solution}$$

$$[\text{Similarly, for 40\% stock solution: } c \text{ ml} = (x\%)(10 \text{ ml})/40\% = (x)(0.25)\text{ml}]$$

d ml: Calculate the volume of water required for the desired total monomer concentration with the following formula:

$$d \text{ ml} = (40 \text{ ml} - 10 \text{ ml} - 100 \text{ µl} - 10 \text{ µl}) - c \text{ ml} = 29.89 \text{ ml} - c \text{ ml}$$

The volume of the stacking gel should be one-and-one-half to twice the sample volume to be electrophoresed. Sample volumes should range from 200 µl to 1 ml.

Section 9

A Guide to Preparative Native-PAGE

9.1 Introduction

Conventional gel electrophoresis buffer systems and media are used with the Mini Prep Cell to separate individual components from their nearest contaminants. This guide describes a method for selecting the best non-denaturing PAGE system to isolate a particular protein with the Mini Prep Cell.

Native PAGE Theory

Preparative native PAGE is a technique for high yield purification of biologically active molecules. In contrast to SDS-PAGE where proteins migrate according to size only, the mobilities of proteins in native PAGE systems depend on both their charges and sizes. There is no single electrophoresis buffer system that will optimally purify all native proteins. When selecting conditions for the purification of a native protein there are some basic parameters to consider: the pI of the protein under investigation, and the pH of the electrophoresis buffer system.

In preparative native PAGE, the most important consideration for optimum resolution of a protein is the pH of the electrophoresis buffer. The pH of the electrophoresis buffer system must be within the pH range over which the protein under study is stable and retains its biological activity. In addition, the pH of the chosen buffer system must impart sufficient charge to the protein for it to move through the gel at a reasonable rate during the run.

Changes in pH alter the charges (and shapes) of proteins. Therefore, during native PAGE, the pH of the electrophoresis buffer will affect the migration rates and resolution patterns of proteins in the sample. For example, a buffer with an alkaline pH value relative to the pI of a particular protein will impart net negative charge to the protein. In such a buffer system, the protein migrates toward the positive electrode (anode). Electrophoresis buffers with acidic pH values relative to the pI of a protein impart net positive charge to the protein so that it migrates toward the negative electrode (cathode). A buffer with a pH value identical to the pI of a protein results in net neutral charge on the protein and it will not move at all in an electric field.

In native PAGE, protein mobilities are best modified by the buffer's pH. Electrophoresis buffers with pH values close to the pI of the protein of interest will theoretically provide the best resolution. However, the resultant migration rate may be too slow for elution from the preparative gel column. Conversely, buffers with pH values farther away from the pI of the protein of interest result in faster migration rates, but, with a loss of resolution. The choice of pH becomes a compromise between separation and speed.

Once the native protein is purified, an enzyme assay or immunoblot can be used to identify the specific location of the protein in a slab-gel or in the fractions collected from the Mini Prep Cell. Analysis by SDS-PAGE can be used to confirm the resolution and purity. Silver stained SDS-PAGE gels will demonstrate the presence of any contaminating proteins.

9.2 How to Choose Native-PAGE Systems

Discontinuous Buffer Systems

The discontinuous buffer system of Ornstein-Davis (Tris/chloride/glycine) should be the first non-denaturing gel system tried.¹ Detailed protocols are provided in Section 9.3 for using this system. An advantage of discontinuous systems for dilute protein solutions is the use of

stacking gels to concentrate the sample. However, the stacking phenomena encountered in discontinuous systems can cause aggregation of some proteins and this can severely interfere with resolution.

The pH attained in the resolving gel of the Ornstein-Davis system approaches pH 9.5, which may be outside the range of stability for some proteins. Alternative discontinuous buffer systems derived for preparative work can be found in an article by Chrambach and Jovin.² The electrophoresis buffers described in this article span the pH range from pH 3 to pH 10.

Continuous Buffer Systems

If discontinuous systems cannot be used because of stacking-induced aggregation, a continuous buffer system will be required. In continuous systems the same buffer is used in the upper and lower electrode chambers and in the gel. McLellan describes various continuous buffer systems from pH 3.8 - 10.2 which can be tried. See Section 9.4 for preparation of these continuous buffers.³

For in depth discussions of electrophoresis and the distinctions between continuous and discontinuous electrophoresis systems, consult references 4-6 in Section 9.5.

Optimizing Conditions for Preparative Native-PAGE

Conditions for purification of native proteins with the Mini Prep Cell should first be optimized on a small scale using mini-slab gels. It is recommended that the procedures described in this guide be repeated for each new sample to be purified. Any optimization procedure should be carried out using the same crude or partially purified protein sample as will be applied to the Mini Prep Cell. Figure 9.1 presents an overview of the optimization procedure.

Isolating individual proteins by preparative native PAGE can be simplified by partially purifying the sample using preparative isoelectric focusing or chromatography before electrophoresis in the Mini Prep Cell.

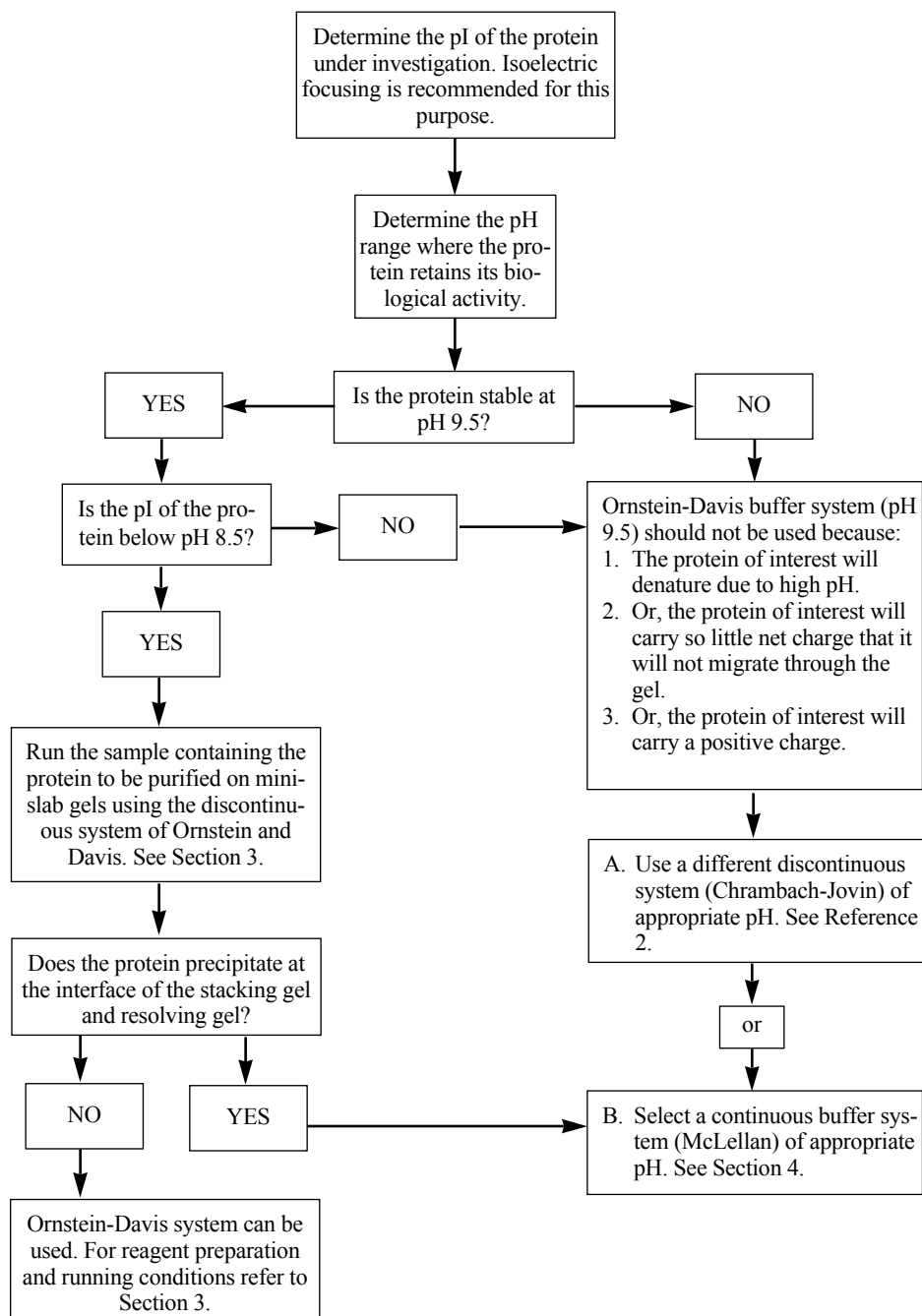


Fig. 9.1. Flow chart for selecting a native electrophoresis buffer system.

9.3 Discontinuous Native-PAGE

Acrylamide Concentration – Gel Pore Size

By convention, polyacrylamide gels are characterized by the figures (%T/%C), where %T is the weight percentage of total monomer including crosslinker (in g/100 ml) and %C is the proportion of crosslinker as a percentage of total monomer. For both the analytical gels and the preparative gels use 2.67% N, N'-methylene-bis-acrylamide crosslinker (Premixed acrylamide:bis in the ratio 37.5:1 can also be used). The total monomer concentration determines the pore size of the gel and is referred to as %T.

Resolution of native proteins during electrophoresis can be affected by changing the pore size of the gel. This change in pore size is accomplished by changing the amount of acrylamide (%T) in the gel. As the pore size changes, the change in mobility differs for each protein in the sample mix. The result is that the separation pattern can be altered by changing the acrylamide concentration.

The optimum gel concentration for preparative work is determined empirically as the one that best separates the protein of interest from its contaminants. In native preparative PAGE, gels with large pores are preferred because of the relatively high migration rates they allow. For discontinuous native PAGE in the Mini Prep Cell, gels between 4% and 10%T/2.67%C provide optimal resolution. Gel lengths of 4-5 cm are sufficient to resolve most proteins. Determination of the optimum acrylamide concentration is accomplished using mini-slab gels as follows.

Acrylamide Concentration – Optimization Procedure

Selection of the optimum gel concentration begins with running the sample in a series of analytical mini-gels composed of 4%, 6%, 8%, and 10% acrylamide. When the marker dye reaches the bottoms of the gels, the runs are terminated and the gels are stained. In each gel, the separation of the protein of interest relative to its contaminants is assessed. The gel with the lowest acrylamide concentration that gives the best resolution is the one to use for native preparative PAGE.

A graphical way to determine the optimum acrylamide concentration from these analytical gels is by comparing the relative mobilities (R_f) of the protein of interest and its nearest contaminant. (To calculate R_f values see Section 4.1). To achieve this, plot the relative mobilities of the protein of interest and its nearest contaminant, as measured in each gel, against acrylamide percentage. From this graph, choose the lowest acrylamide concentration that gives the greatest difference in R_f between these two proteins. This is the %T to use for preparative native-PAGE in the Mini Prep Cell.

Polyacrylamide Gel Recipes

Prepare a 30% Acrylamide Stock Solution:

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

Acrylamide - 146.0 g

N'N'-bis-methylene-acrylamide (Bis) - 4.0 g

Dissolve in 350 ml deionized water then adjust to 500 ml with deionized water. Filter and store at 4 °C in the dark.

(Prewighed Acrylamide/Bis 37.5:1 mixture or 40% Acrylamide/Bis Stock solution can be substituted. Calculate the volume of a 40% stock solution required to make the desired gel).

Preparing Ornstein-Davis Native Electrophoresis Buffer Solutions

Resolving (Separating) Gel Buffer (1.5 M Tris-HCl pH 8.8)

Dissolve 27.23 grams Tris base in approximately 80 ml deionized water.

Adjust to pH 8.8 with 6 N HCl.

Make to 150 ml with deionized water and store at 4 °C.

Stacking Gel Buffer (0.5 M Tris-HCl, pH 6.8)

Dissolve 6 grams Tris base in approximately 60 ml deionized water.

Adjust to pH 6.8 with 6 N HCl.

Make to 100 ml with deionized water and store at 4 °C.

Sample Buffer (0.0625 M Tris-Cl, pH 6.8, 25% Glycerol, 0.012% Bromophenol Blue)

Deionized water - 4.8 ml

0.5 M Tris-HCl, pH 6.8 - 1.0 ml

Glycerol - 2.0 ml

0.5% Bromophenol-blue (optional) - 0.2 ml

Total volume - 8.0 ml

10x Electrode Running Buffer, pH 8.3 (Makes 1 Liter)

Tris base - 30.3 g

Glycine - 144.0 g

Dissolve and adjust to 1,000 ml with deionized water. DO NOT adjust pH with acid or base.

To make 1x Electrode Running Buffer (1 Liters; 0.025 M Tris, 0.192 M Glycine, pH 8.3)

Dilute 100 ml 10x electrophoresis buffer (pH 8.3) with 900 ml deionized water. Do not adjust pH with acid or base.

Prepare Ornstein-Davis Acrylamide Gels

Use the following table to prepare acrylamide gels (both analytical and preparative). The amounts listed for the components in the table below are based on a total volume of 10 ml.

| %T | Deionized H ₂ O | Gel Buffer* | Acrylamide / Bis solution 30% stock (37.5:1) |
|----|----------------------------|-------------|---|
| | (ml) | (ml) | (ml) |
| 4 | 6.15 | 2.50 | 1.33 |
| 5 | 5.80 | 2.50 | 1.67 |
| 6 | 5.55 | 2.50 | 2.00 |
| 7 | 5.15 | 2.50 | 2.33 |
| 8 | 4.80 | 2.50 | 2.67 |
| 9 | 4.45 | 2.50 | 3.00 |
| 10 | 4.15 | 2.50 | 3.33 |

*Resolving Gel buffer - 1.5 M Tris-HCl 8.8

*Stacking Gel buffer - 0.5 M Tris-HCl 6.8

Prior to pouring a gel, induce acrylamide polymerization by adding the following catalyst solutions. Amounts are per 10 ml gel volume.

| | Catalyst | |
|--------------------|------------|----------|
| | 10% APS ** | TEMED ** |
| Analytical | | |
| Resolving Gel | 50 µl | 5 µl |
| Stacking Gel | 50 µl | 10 µl |
| Preparative | | |
| Resolving Gel | 25 µl | 2.5 µl |
| Stacking Gel | 50 µl | 10 µl |

****Note:** To slow the rate of polymerization and avoid temperature induced inconsistencies in then gel, different amounts of catalyst are used for preparative gels and analytical gels. To make 10% APS, dissolve 100 mg in 1 ml of deionized water.

Running Conditions for Discontinuous Native-PAGE

1. Run the Mini Prep Cell at 1 watt constant power. Cooling the lower buffer and (or) running the gel in the cold room will help to maintain the biological activity of some proteins.
2. An elution flow rate of 0.75 µl to 100 µl/minute is recommended. Fractions should be collected following elution of the ion front.

- 3 Protein migration rates of 1.0- 2.0 cm/hour correspond to approximate elution times of 3-6 hours for a 5 cm gel.
4. The migration rates of proteins run in Ornstein-Davis gels at 1 watt constant power will approximate those shown in the following table.

| R_f | Migration rate |
|----------------------|-----------------------|
| 1.0 | 30 minutes/cm gel |
| 0.8 | 40 minutes/cm gel |
| 0.6 | 50 minutes/cm gel |
| 0.45 | 60 minutes/cm gel |

R_f values for specific proteins are obtained from the mini-gels that were run to optimize conditions for the Mini Prep Cell. To calculate R_f values for specific proteins use this formula:

$$R_f = \frac{\text{Distance that the protein of interest migrated}}{\text{Distance that the tracking dye migrated}}$$

The R_f value obtained from a mini gel can be used to estimate when a protein will elute from the Mini Prep Cell when the same concentration of acrylamide is used in both the mini gel and the preparative gel. R_f values below 0.45 may result in excessive band broadening and loss of resolution.

$$\text{Elution Time for a Protein} = \frac{\text{Elution time for the tracking dye}}{R_f \text{ of the Protein}}$$

9.4 Continuous Native-PAGE

Selection of Continuous Buffer Systems

In continuous systems the same buffer is used in the electrode chambers and in the gels. Since stacking gels are not commonly employed, proteins migrate in bands at least as wide as the applied sample. Therefore, the sample volume must be kept at a minimum. The mobilities of proteins in continuous systems are dictated primarily by pH rather than by sieving through the polyacrylamide gel. For this reason, 6% polyacrylamide gels are recommended for most applications. For very large proteins 4% or 5% gels may be used.

1. Use the Table on page 27 to prepare electrophoresis buffers within the operating pH range of the protein under investigation. Make sure the acidic and basic components used are compatible with the protein under investigation.
2. It is difficult to predict the migration rate of proteins in native buffer systems without preliminary analysis. For a chosen buffer system, determine if the protein of interest is negatively or positively charged.
 - A. If the pI of the protein of interest is < pH of the buffer system, the protein is negatively charged and will migrate to the anode.
 - B. If the pI of the protein of interest is > pH of the buffer system, the protein is positively charged and will migrate towards the cathode. In this case make sure to reverse the electrodes to the power supply to ensure migration into the gel.

3. Prepare 6%T/2.67%C mini-gels with the selected buffers. See Section 9.3.5 for preparation of a 6% acrylamide gel. Electrophoresis is carried out at approximately 200 volts for about one hour.
4. Mini-gels should be silver stained to determine the level of contamination of the protein of interest. The buffer system of the gel showing the best resolution and exhibiting a migration rate for the protein of interest of at least 0.5 mm/minute is ideal for the preparative gel.

Buffers used for continuous systems are not limited to those listed in the following section. Virtually any buffer can be used if it proves appropriate for the protein under investigation.

Continuous Electrophoresis Buffers

McLellan describes various continuous buffer systems from pH 3.8 to pH 10.2.³ Use the Table below to prepare 5x continuous non-denaturing PAGE electrophoresis buffers. Add both the acidic and basic components to 1 liter of water. Do Not adjust pH with acid or base. If the final pH is outside the listed range discard the buffer and remake.

| Buffer pH \pm 0.1 | Basic component and MW | 5X solution g/L or ml/L | Acidic component and MW | 5X solution ml/L or gm/L |
|------------------------|------------------------------|-------------------------------|-------------------------------|--------------------------------|
| 3.8 | Beta-Alanine 89.09 mw | 13.36 g/L | Lactic Acid 85% soln. | 7.45 ml/L |
| 4.4 | Beta-Alanine 89.09 mw | 35.64 g/L | Acetic Acid 17.4 M | 11.5 ml/L |
| 4.8 | GABA 103.1 mw | 41.24 g/L | Acetic Acid 17.4 M | 5.75 g/L |
| 6.1 | Histidine 155.2 mw | 23.28 g/L | MES 195.2 mw | 29.5 g/L |
| 6.6 | Histidine 155.2 mw | 19.4 g/L | MOPS 209.3 mw | 31.4 g/L |
| 7.4 | Imidazole 68.08 | 14.64 g/L | HEPES 238.33 mw | 41.7 g/L |
| 8.1 | Tris 121.14 mw | 19.38 g/L | EPPS 252.2 mw | 37.85 g/L |
| 8.7 | Tris 121.14 mw | 30.29 g/L | Boric Acid 61.83 mw | 7.73 g/L |
| 9.4 | Tris 121.14 mw | 36.34 g/L | CAPS 221.3 mw | 44.26 g/L |
| 10.2 | Ammonia 14.8 M | 12.5 ml/L | CAPS 221.3 mw | 22.13 g/L |

To make 1 liter of 1x electrophoresis buffer, dilute 200 ml of 5x buffer with 800 ml deionized water. The final concentrations of buffer components will be:

| Buffer pH | Basic Component | Acidic component |
|-----------|--------------------|-------------------|
| 3.8 | 30 mM Beta-Alanine | 20 mM Lactic Acid |
| 4.4 | 80 mM Beta-Alanine | 40 mM Acetic Acid |
| 4.8 | 80 mM Gaba | 20 mM Acetic Acid |
| 6.1 | 30 mM Histidine | 30 mM MES |
| 6.6 | 25 mM Histidine | 30 mM MOPS |
| 7.4 | 43 mM Imidazole | 35 mM HEPES |
| 8.1 | 32 mM Tris | 30 mM EPPS |
| 8.7 | 50 mM Tris | 25 mM Boric Acid |
| 9.4 | 60 mM Tris | 40 mM CAPS |
| 10.2 | 37 mM Ammonia | 20 mM CAPS |

Prepare Resolving Gels

As protein mobilities are best modified by pH, continuous non-denaturing PAGE systems use relatively large pore size gels. Generally 4-6 cm long gels are sufficient for optimum resolution in the Mini Prep Cell.

(Per 10 ml acrylamide monomer solution)

| %T | Deionized H ₂ O (ml) | Continuous Buffer* (ml) | Acrylamide / Bis solution 30% stock (37.5:1) |
|----|---------------------------------|-------------------------|--|
| 4 | 6.65 | 2.00 | 1.33 |
| 5 | 6.30 | 2.00 | 1.67 |
| 6 | 6.05 | 2.00 | 2.00 |

*In continuous systems the same buffer is used in the upper and lower electrode chambers and in the gels.

Prior to pouring a gel, induce acrylamide polymerization by adding the following catalyst solutions. Amounts are per 10 ml gel volume.

| Catalyst | 10% APS** | TEMED** |
|---------------------------|-----------|---------|
| Analytical Resolving Gel | 50 µl | 10 µl |
| Preparative Resolving Gel | 50 µl | 10 µl |

NOTE: Below pH 6, TEMED becomes less effective as a catalyst. Between pH 4 and pH 6, increasing the concentration of TEMED 5-fold to 10-fold will polymerize the gel.

Sample Preparation

Sample buffer for continuous native-PAGE is a dilution of the electrophoresis buffer. Tracking dyes are generally not used. The concentration of the sample buffer is generally 1/10 that of the running buffer. Glycerol is added to the sample buffer to a final concentration of 20%.

Important: Keep the protein sample as concentrated as possible since there are no stacking gels used with these buffer systems. Bands will be at least as wide as the sample that is loaded onto the preparative gel.

Running Conditions for the Mini Prep Cell

For continuous buffer systems, run the gels at 1 watt constant power (approximately 3 mA and 300 volts). Cooling the lower buffer and (or) running the Mini Prep Cell in a cold room may help to maintain the biological activity of some proteins.

The elution buffer flow rate for the Mini Prep Cell should be set to 75 μ l to 100 μ l/minute. Native proteins migrate at individual velocities dependent on the pH of the buffer system used. Therefore, it is difficult to predict exactly in which fractions the protein of interest will elute. An enzyme assay or immunoblot can be used to identify the specific location of the protein in a slab-gel or in the fractions collected from the Mini Prep Cell. Analysis by SDS-PAGE can be used to confirm the resolution and purity.

9.5 References

1. L. Ornstein and B.J. Davis, *Anal. NY Acad. Sci.*, **121**, p.321, (1964).
2. A. Chrambach and T.M. Jovin, *Electrophoresis*, **4**, 190-204, (1984).
3. T. McLellan, *Analytical Biochemistry*, 126, 94-99, (1982).
4. B.D. Hames, (1990), in *Gel Electrophoresis of Proteins: A Practical Approach*, (ed. D. Rickwood and B.D. Hames), Oxford University Press, New York.
5. A.T. Andrews, (1986), in *Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications*, Clarendon Press, Oxford.
6. R.C. Allen, C.A. Saravis, H.R. Maurer, (1984), in *Gel Electrophoresis and Isoelectric Focusing of Proteins: Selected Techniques*, de Gruyter, New York.

Section 10 Product Information

| Catalog Number | Product Description |
|--|--|
| 170-2926 | Model 491 Prep Cell , Buffer Recirculation Pump, and reagent starter kit, 100/120 VAC |
| 170-2927 | Model 491 Prep Cell , Buffer Recirculation Pump, and reagent starter kit, 220/240 VAC |
| 170-2908 | Mini Prep Cell |
| <i>Mini Prep Cell Replacement Parts</i> | |
| 170-2909 | Gels Tubes , 2 |
| 170-2940 | Thumb Screws , 4 |
| 170-2913 | Sample application/Purge kit |
| 170-2947 | Peristaltic Pump Kit |
| 170-2948 | Elution Frit w/3.5 K Dialysis membranes , 5 |
| 170-2911 | Elution Frit w/6 K Dialysis membranes , 5 |
| 170-2912 | Harvest Ring Assembly , includes elution collection tube |
| <i>Recommended Reagents</i> | |
| 161-0122 | Premixed Acrylamide/Bis, 37.5:1 mixture (2.67% C) , 30 g |
| 161-0125 | Premixed Acrylamide/Bis, 37.5:1 mixture (2.67% C) , 150 g |
| 161-0148 | 40% Acrylamide/Bis Solution, 37.5:1 mixture (2.67% C) , 500 ml |
| 161-0158 | 30% Acrylamide/Bis Solution, 37.5:1 mixture (2.67% C) , 500 ml |
| 161-0700 | APS , 10 g |
| 161-0201 | Bis , 50 g |
| 161-0800 | TEMED , 5 ml |
| 161-0755 | Premixed Buffer, 10x Tris/glycine/SDS , 6 x 1L |
| 161-0300 | SDS , 25 g |
| <i>Auxiliary Instruments</i> | |
| 165-5056 | PowerPac 3000 Power Supply , 110/120 VAC |
| 165-5057 | PowerPac 3000 Power Supply , 220/240 VAC |
| 165-5054 | PowerPac 300 Power Supply , 110/120 VAC |
| 165-5054 | PowerPac 300 Power Supply , 220/240 VAC |
| 731-8140 | Peristaltic Pump: Model EP-1 Econo Pump , 100/120 VAC |
| 731-8142 | Peristaltic Pump: Model EP-1 Econo Pump , 220/240 VAC |
| 731-8122 | Model 2110 Fraction Collector , 100/120 VAC |
| 731-8120 | Model 2110 Fraction Collector , 220/240 VAC |
| 731-8123 | Model 2128 Fraction Collector , 110 V |
| 731-8124 | Model 2128 Fraction Collector , 220 V |



**Bio-Rad
Laboratories**

**Life Science
Group**

2000 Alfred Nobel Drive
Hercules, California 94547
Telephone (510) 741-1000
Fax: (510) 741-1060

Eastern Regional Office, 85A Marcus Dr., Melville, New York 11747 • Phone (516) 756-2575 • Fax (516) 756-2594
Australia, Bio-Rad Laboratories Pty Limited, Unit 11, 112-118 Talavera Rd P.O. Box 371, North Ryde, NSW 2113 • Phone 02-805-5000 • Fax 02-805-1920
Austria, Bio-Rad Laboratories Ges.m.b.H., Auhofstrasse 78D, 1130 Wien • Phone (1) 877 89 01 • Fax (1) 876 56 29
Belgium, Bio-Rad Laboratories S.A./N.V., Begoniastraat 5, 9810 Nazareth Eke • Phone 09-385 55 11 • Fax 09-385 65 54
Canada, Bio-Rad Laboratories (Canada) Ltd., 5671 McAdam Road, Mississauga, Ontario L4Z 1N9 • Phone (905) 712-2771 • Fax (905) 712-2990
China, Bio-Rad Laboratories, 14, Zhi Chun Road, Hai Dian District, Beijing 100088 • Phone (01) 2046622 • Fax (01) 2051876
Denmark, Bio-Rad Laboratories, Symbion Science Park, Fruebjergvej 3, DK-2100 Copenhagen • Phone 45-39 17 99 47 • Fax 45-39 27 16 98
France, Bio-Rad S.A., 94/96 rue Victor Hugo, B.P. 220, 94 203 Ivry Sur Seine Cedex • Phone (1) 49 60 68 34 • Fax (1) 46 71 24 67
Germany, Bio-Rad Laboratories GmbH, Heidemannstraße 164, D-80939 München/Postfach 450133, D-80901 München • Phone 089 31884-0 • Fax 089 31884-100
India, Bio-Rad Laboratories, C-248 Defence Colony, New Delhi 110 024 • Phone 91-11-461-0103 • Fax 91-11-461-0765
Italy, Bio-Rad Laboratories S.r.l., Via Cellini, 18/A, 20090 Segrate Milano • Phone 02-21609 1 • Fax 02-21609-399
Japan, Nippon Bio-Rad Laboratories, Sumitomo Seimei Kachidoki Bldg, 3-6 Kachidoki, 5-Chome, Chuo-Ku, Tokyo 104 • Phone 03-3534-7665 • Fax 03-3534-8497
The Netherlands, Bio-Rad Laboratories B. V., Fokkerstraat 10, 3905 KV Veenendaal • Phone 0318-540666 • Fax 0318-542216
New Zealand, Bio-Rad Laboratories Pty Ltd., P. O. Box 100-051, North Shore Mail Centre, Auckland 10 • Phone 09-443 3099 • Fax 09-443 3097
Pacific, Bio-Rad Laboratories, Unit 1111, 11/F., New Kowloon Plaza, 38, Tai Kok Tsui Road, Tai Kok Tsui, Kowloon, Hong Kong • Phone 7893300 • Fax 7891257
Singapore, Bio-Rad Laboratories (Singapore) Ltd., 464 Siglap Road, #01-02 Flamingo Valley, Singapore 1545 • Phone (65) 4432529 • Fax (65) 4421667
Spain, Bio-Rad Laboratories, S. A. Avda Valdelaparra 3, Pol. Ind. Alcobendas, E-28100 Alcobendas, Madrid • Phone (91) 661 70 85 • Fax (91) 661 96 98
Sweden, Bio-Rad Laboratories AB, Gårdsvägen 7D, Box 1276, S-171 24 Solna • Phone 46-(0)8-735 83 00 • Fax 46-(0)8-735 54 60
Switzerland, Bio-Rad Laboratories AG, Kanalstrasse 17, Postfach, CH-8152 Glattbrugg • Phone 01-809 55 55 • Fax 01-809 55 00
United Kingdom, Bio-Rad Laboratories Ltd., Bio-Rad House, Maylands Avenue, Hemel Hempstead, Herts HP2 7TD • Free Phone 0800 181134 • Fax 01442 259118