Model 491 Prep Cell

Instruction Manual

For Technical Service call your local Bio-Rad office or in the U.S. call 1-800-424-6723.

BIO-RAD

Note

To ensure the best performance from the Model 491 prep cell, become fully acquanted with these operating instructions before using the cell to transfer samples. Bio-Rad recommends that you first read these instructions carefully. Then assemble and disassemble the cell completely without transferring sample. After these preliminary steps, you should be ready to transfer a sample.

Bio-Rad also recommends that all Model 491 prep cell components and accessories be cleaned with a suitable laboratory cleaner (such as Bio-Rad cleaning concentrate, catalog number 161-0722) and rinsed thoroughly with distilled water, before use.

Model _____

Catalog No. _____

Date of Delivery _____

Serial No. _____

Invoice No. _____

Purchase Order No. _____

Warranty

Bio-Rad Laboratories' Model 491 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 of charge. 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.
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.

Table of Contents

Section 1
General Information

1.1 Introduction

The Model 491 prep cell* is designed to purify proteins or nucleic acids from complex mixtures by continuous-elution electrophoresis. Conventional gel electrophoresis buffer systems and media are used with the prep cell.

During a run, samples are electrophoresed through a cylindrical gel. As molecules migrate through the gel matrix, they separate into ring shaped 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 of a specially designed gasket. The unique design of the gasket results in an even flow of buffer into the elution frit. Buffer is drawn radially inward to an elution tube in the center of the cooling core. Purified molecules are drawn up through the elution collection tube at the center of the cooling core by a peristaltic pump. The peristaltic pump drives separated proteins through a UV monitor (optional) to a fraction collector (Bio-Rad's Econo™ system).

To assure that separated molecules migrate in compact, parallel bands, temperature gradients across the gel are minimized. The temperatures of the internal and external surfaces of the gel are equalized by continuously pumping lower electrophoresis buffer through the central cooling core by means of the buffer recirculation pump.

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 Model 491 prep cell.

1.2 Accessory Equipment

Power supply — 500 volt
Warning: Use only power supplies with isolated ground such as Bio-Rad's PowerPac™ HV or PowerPac Universal

Buffer recirculation pump — (provided with the prep cell

Peristaltic pump (elution pump)*

Fraction collector*

UV monitor

Chart recorder**

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

** The use of a reliable fraction collector is essential for the isolation of the desired component of the sample. It may also be convenient to monitor elution by UV absorbance. To simplify setup and operation of accessory equipment, we recommend use of Econo system low pressure chromatography components, including peristaltic pump, fraction collector, UV monitor, and chart recorder.

1.3 Specifications

Construction

Upper buffer chamber	acrylic
Lower buffer chamber	acrylic
Electrodes	platinum, 0.010 inch diameter
Lid	acrylic
Gel tube assembly	glass/acrylic
Elution chamber base	acrylic
Elution frit	polyethylene
Support frit	polyethylene
Cooling core	glazed alumina
Elution tube	borosilicate glass, 0.06” ID
Casting stand	acrylic
Shipping weight	6 lb
Overall size	7 in. diameter x 14 in. high
Voltage limit	500 volts
Current limit	40 milliamperes
Power limit	20 watts
Cooling buffer flow rate	100 ml/min
Elution buffer flow rate	60 ml/hour
Upper electrophoresis buffer volume	300–600 ml
Elution buffer chamber volume	900 ml
Lower electrophoresis buffer volume	2–3 L

1.4 Chemical Compatibility

The Model 491 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 Model 491 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 HV power supply. The PowerPac universal power supply may also be used. The maximum specified operating parameters for the Model 491 prep cell are:

500 VDC ⎓	maximum operating voltage
40 mA	maximum operating current
20 W	maximum operating power limit

Current to the Model 491 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 buffer recirculation pump is also ground isolated as should be any pump used with this cell. During normal operation, the buffer in the lower buffer chamber is circulated through the cooling core and routed back into the system via the buffer recirculation pump. The buffer flowing through the tubing and the pump is electrically active. For this reason handle the tubing carefully while the power supply is on. **Do not touch any exposed liquid when the power supply is on.** Tube connections should be made with the power supply turned off. Both the recirculation pump and 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. As a result it is the user's responsibility to always excercise 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.

Important

This Bio-Rad instrument is designed and certified to meet IEC1010-1* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the IEC1010-1 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

*IEC1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

Section 2
Description of Major Components

2.1 Model 491 Prep Cell Components

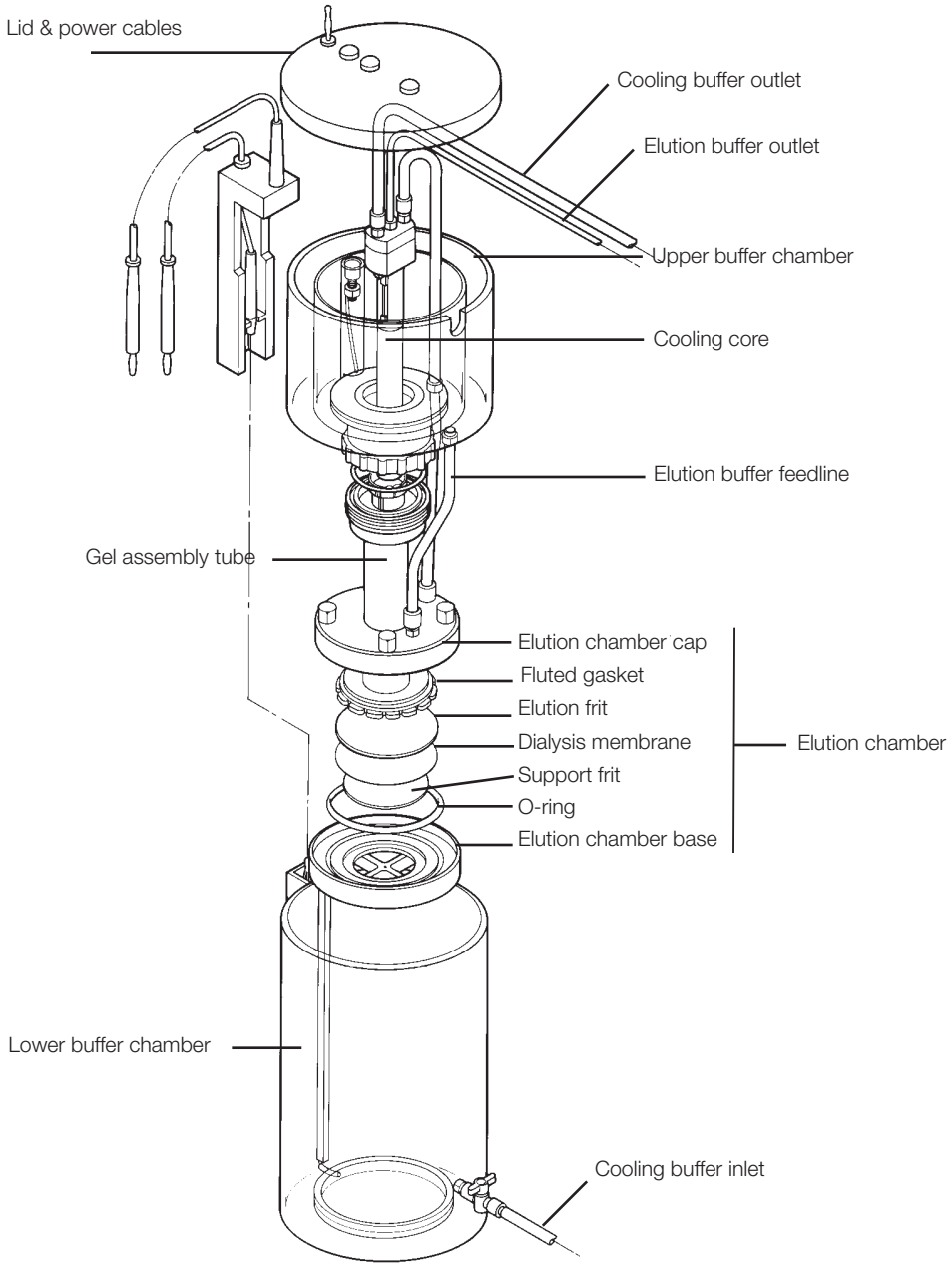


Fig. 1. Exploded view of the Model 491 prep cell.

2.2 Lower and Upper Buffer Chamber

The lower buffer chamber forms a stable base for the unit. It houses the anode and contains the lower electrophoresis buffer. The upper buffer chamber holds the upper electrophoresis buffer and the elution buffer, and houses the cathode.

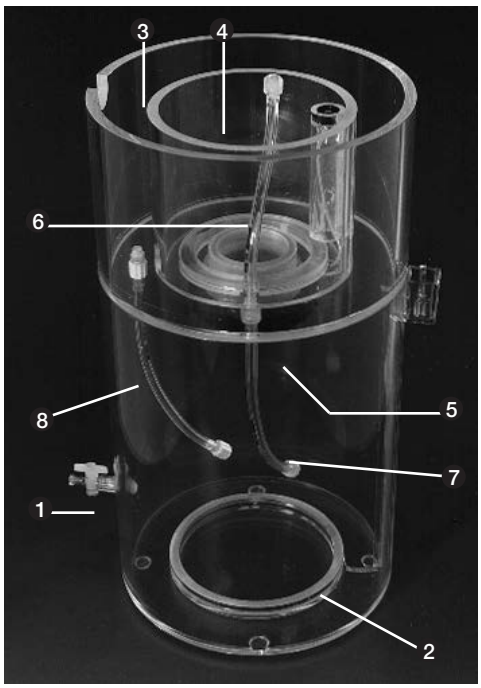


Fig. 2. Upper and lower buffer chamber. Lower buffer chamber: Stopcock for lower electrophoresis buffer inlet (1), and anode (2). Upper buffer chamber: Elution buffer reservoir (3), upper electrophoresis buffer reservoir (4), ring nut (5), cooling buffer line (6), cooling buffer feedline (7), and elution buffer feedline (8).

2.3 Cooling Core

The cooling core extends to the bottom of the graduated gel tube and houses the elution tube in its center. Cooling is recommended during polymerization as well as during electrophoresis.

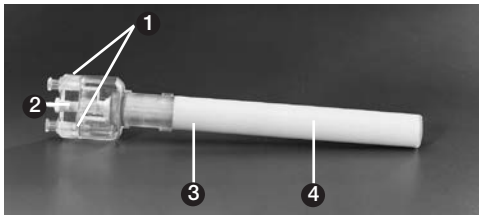


Fig. 3. Cooling core. Cooling buffer ports (1), elution buffer outlet (2), cooling core placement guides (3), and glazed ceramic cooling core (4).

2.4 Gel Tube Assembly

The gel tube assembly holds both the gel and the cooling core. The elution chamber cap and the gasket mounted on the graduated gel column make up the upper part of the elution chamber. Two gel tube assemblies are provided with the Model 491 prep cell: 28 mm ID and 37 mm ID. See Section 4.4 for selecting the appropriate gel tube size for specific applications.

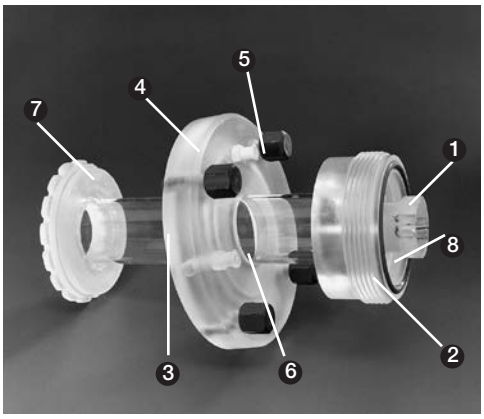


Fig. 4. Gel tube assembly. Cooling core collar (1), upper reservoir attachment, i.e. threaded connector (2), graduated gel column (3), elution chamber cap with thumb screws (4), elution buffer inlet port (5), cooling buffer port (6), gasket (7), and O-ring (8).

2.5 Elution Chamber Base

The elution chamber base with the support frit holds the elution frit and the dialysis membrane directly beneath the gel. The dialysis membrane provided with the Model 491 prep cell has a molecular weight cut off of 6,000 daltons. A continuous flow of elution buffer is directed through the channels of the elution chamber gasket to the perimeter of the elution frit. As bands migrate off the gel, they are washed to the center of the frit, up through the elution tube in the center of the cooling core, and out to the peristaltic pump, UV detector (optional), and fraction collector. (The tracking dye, bromophenol blue (M_r 691), does not pass through the dialysis membrane. The flow of elution buffer through the elution frit overcomes the force of electrophoresis in the downward direction.)

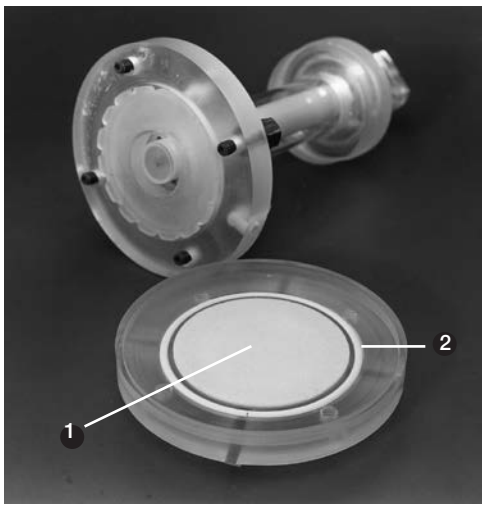


Fig. 5. Elution chamber base and tube assembly. Elution frit (1) and large O-ring (2).

2.6 Casting Stand

Gels are cast with the gel tube assembly mounted directly on the casting stand. The casting stand ensures that gels have perfectly flat lower surfaces. Inserting a spatula in the gel-release slot facilitates the removal of the gel from the casting stand after polymerization by allowing air to enter beneath the gasket and the gel.

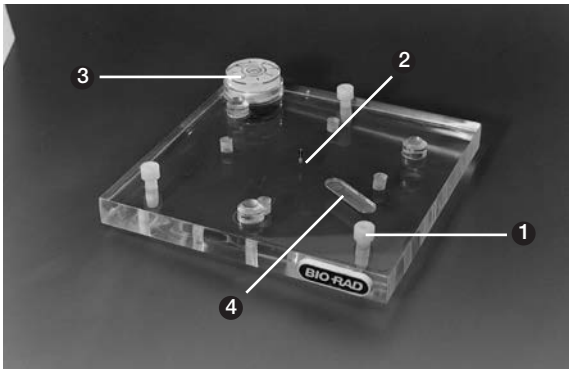
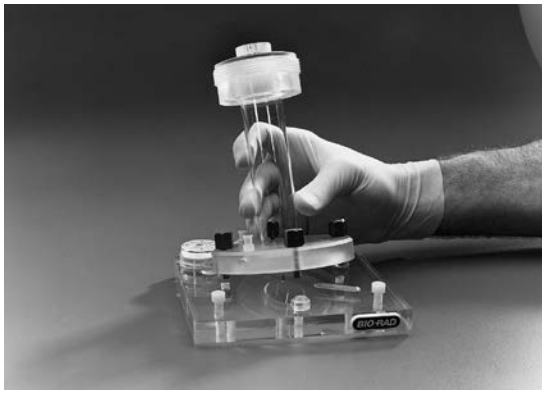


Fig. 6. Casting stand. Leveling feet (1), center pin (2), leveling bubble (3), and gel release slot (4).

Section 3
Assembly and Operation

3.1 Casting the Preparative Gel

1. Place the gel tube assembly on the casting stand, aligning the four screws on the acrylic plate with the holes in the casting stand. Secure the gel tube assembly with the four screws; hand tightening is sufficient. Level the casting stand with the aid of the leveling bubble using the leveling legs.



2. Insert the cooling core so that the two placement guides slide through the grooves of the cooling core collar in the gel tube assembly and the center pin on the casting stand is inserted in the elution tube of the cooling core. Turn the core 90° until it locks into place. This will prevent any vertical or lateral movement of the cooling core.

3. It is advisable to cool the gel during polymerization. Cooling prevents excess heat accumulation in the interior of the reaction mixture and aids in the formation of uniform gels. To cool, pump room temperature water (or buffer) from an external source through the cooling core. Ensure that cooling is in progress prior to casting the gel.

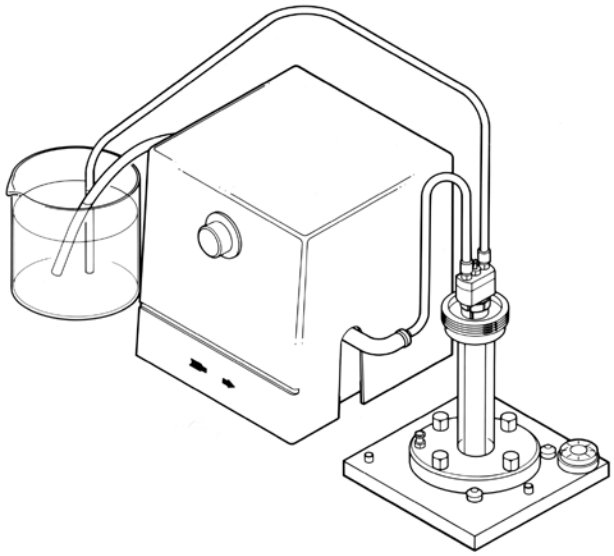


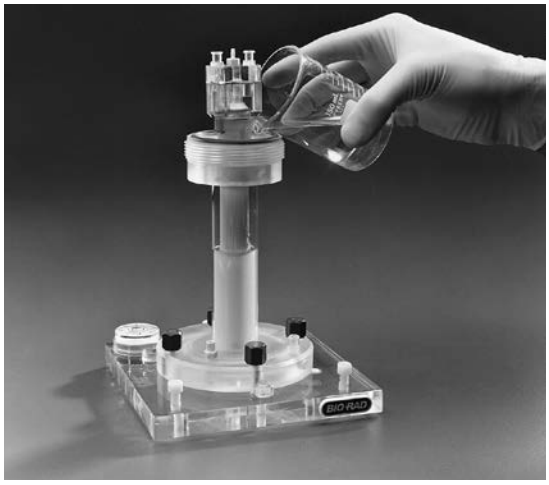
Fig. 7. Diagram of cooling path during polymerization. Cooling of gels during polymerization is recommended. This is accomplished by circulating room temperature buffer between a reservoir and the cooling finger using the buffer recirculation pump.

4. 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, repsectively.

5. Pour the degassed monomer mixture into the gel tube through the gap between the cooling core and the collar of the gel tube assembly. Avoid trapping air bubbles in the gel. Gently tapping the casting stand (with the gel tube assembly mounted to it) against the bench top will help to dislodge trapped air bubbles. Visually inspect the gel for bubbles immediately after pouring the separating-gel solution into the tube.

   Carefully overlay the resolving gel with water-saturated 2-butanol or tert-amyl alcohol using the narrow polytetrafluoroethylene (PTFE) tube affixed to 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 nondenaturing gels, this should be 0.375 M Tris/Cl, pH 8.8 buffer.

6. 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

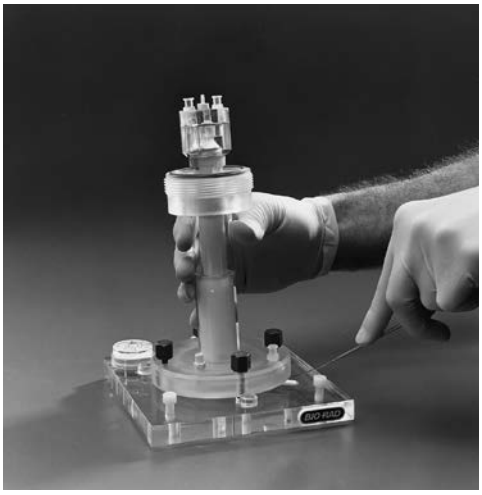
Soak the elution manifold support frit, elution frit, and dialysis membrane in buffer. The frits must be completely wetted prior to use. To ensure removal of entrapped air in the pores of the frits, place the container in which the frits are soaking in a vacuum chamber for approximately 10 minutes. Alternatively, the frits can be soaked in buffer overnight to completely wet them. To maintain the wetting of the frits, store them in buffer.

The dialysis membrane provided with the Model 491 prep cell has a molecular weight cut off of 6,000 daltons. Dialysis membranes with other pore sizes may be substituted for those provided. 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.

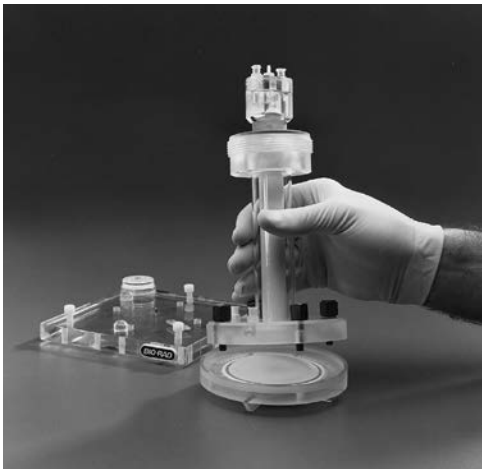
3.3 Assembly of the Elution Chamber

1. Insert the soaked support frit into the base of the elution chamber. The stepped support frit is the thicker of the two frits. Press the frit all the way into the base to form a flat surface on which to place the dialysis membrane. Place the dialysis membrane on the support frit and the elution frit on the dialysis membrane. Press the large elution O-ring in the groove around the perimeter of the base.

2. Decant the stacking gel overlay, rinse the surface of the stacking gel with water, and loosen the four screws holding the column to the casting stand. Carefully remove the gel tube assembly from the casting stand. Insert a spatula into the gel release slot and use it to gently pry the gel tube assembly off the casting stand. Inspect the lower surface of the gel to make sure it is smooth. Trapped air bubbles may cause a pitted gel surface which will result in uneven elution of proteins from the gel. If a pitted or otherwise uneven gel surface is observed, pour a new gel.



3. Place the gel tube assembly containing the gel on the elution chamber base. Align the four screws with the holes in the elution chamber base and hand tighten them.



4. To simplify sample loading, the sample loading guide should be inserted into the space between the cooling core and the gel tube at this time.

3.4 Assembly of Upper and Lower Buffer Chambers

1. Carefully place the upper buffer reservoir on the gel tube assembly and seat it firmly. Make sure the small O-ring is properly seated in the threaded connector.

   The following connections should be made before the black ring nut is tightened. Align and connect the elution buffer feedline (white cap) to the elution buffer reservoir and to the elution port (white port) on the elution chamber. Then connect the recirculation buffer feedline (clear cap) to the port at the bottom of the upper electrophoresis buffer reservoir and to the recirculation buffer port on the elution chamber (clear port).

2. Once the feedlines from the upper buffer chamber are aligned and secured to the connectors on the elution chamber, hand tighten the black ring nut. This procedure will prevent attempting to turn the chamber against the tightened nut. Connect the cooling buffer line (clear cap) already attached inside the upper electrophoresis reservoir with one of the two clear ports on the top of the cooling core to form a small loop. Secure the second cooling buffer line (clear cap) to the other clear port on the cooling core; this line connects to the inlet of the buffer recirculation pump. Connect the small diameter elution tubing to the white elution buffer outlet in the center of the cooling core. Guide both the recirculating buffer line and the elution tubing from the cooling core through the notch at the perimeter of the upper buffer chamber.

3. Fill the upper electrophoresis buffer reservoir (300–600 ml) and the elution buffer reservoir (750 ml) and check that all the lines are properly connected. If leaking is observed, check connections and reconnect where necessary.
4. Thoroughly degas 1,500 ml electrophoresis/elution buffer. With the stopcock of the lower buffer tank closed, fill the lower buffer reservoir with buffer to cover at least the height of gel. The level of lower electrophoresis buffer should be at least 1–2 cm above the level of the gel poured within the tube. Place the whole gel tube assembly into the lower buffer reservoir. See Sections 7 and 9 for buffer recipes.

3.5 Purge the Elution Chamber of Air Bubbles

The elution chamber must be purged of air prior to running the cell. To do this, attach a 50 ml syringe to the elution buffer outlet tubing at the top of the cooling core. Gently pull elution buffer through the elution chamber into the 50 ml syringe. To remove air bubbles trapped in the channels of the gray elution gasket, gently push buffer (not air) back into the elution chamber with a full syringe. As you do this, you will see the air bubbles trapped in the elution chamber exit through the port into the elution buffer reservoir. It may help to lean the prep cell so that the feedline port on the elution chamber is uppermost on the chamber making it easier for bubbles to flow up and out into the elution buffer reservoir.

Release the syringe and attach the elution buffer outlet tubing to a peristaltic pump. Set the elution pump speed to 1 ml/min. Connect the outlet of the pump to a fraction collector.

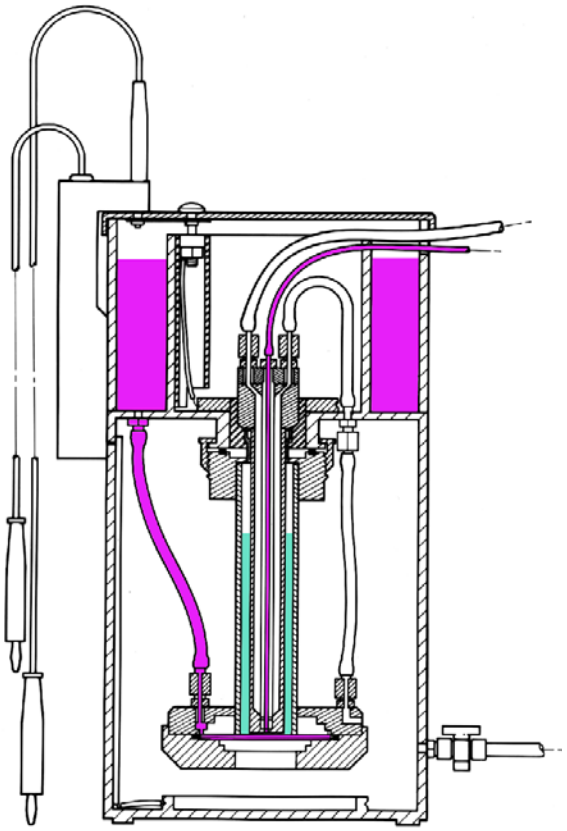


Fig. 8. Elution buffer pathway. Elution buffer is pulled from the elution buffer reservoir to the elution buffer port on the elution chamber cap. The flow is directed through a series of channels in the gray elution chamber gasket to the perimeter of the elution frit. From there, elution buffer and separated proteins emerging from the gel are drawn radially to the center of the elution frit and out through the elution tube in the center of the cooling core. The elution tube is connected to a peristaltic pump, which drives the elution buffer to a detector (optional) and a fraction collector.

3.6 Cooling the Gel

First, determine which setting on the buffer recirculation pump will provide a flow rate of ~100 ml/min. Next, using the tubing provided with the Model 491 prep cell, connect the outlet of the buffer pump to the inlet (stopcock) of the lower buffer chamber. Then connect the tubing exiting from the top of the cooling core to the inlet of the buffer pump. Open the valve and set the pump speed at the maximal setting for a few minutes to purge air from the lines. Then, reset the pump speed to 80–100 ml/min.

For separation of native proteins where colder temperatures are desirable a 2–3 meter length of tubing can be submerged in an ice bath, or an external refrigerator bath. The extra tubing should be placed in line between the buffer recirculation pump outlet and the inlet to the lower buffer chamber. The Model 491 prep cell can also be run in a cold room to keep fractions cold once they have been collected.

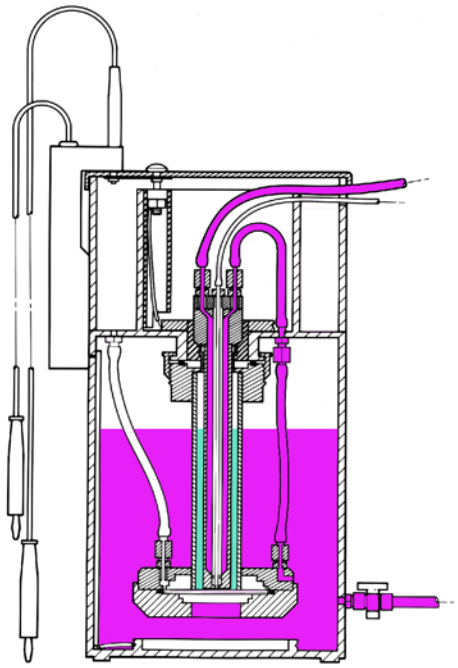
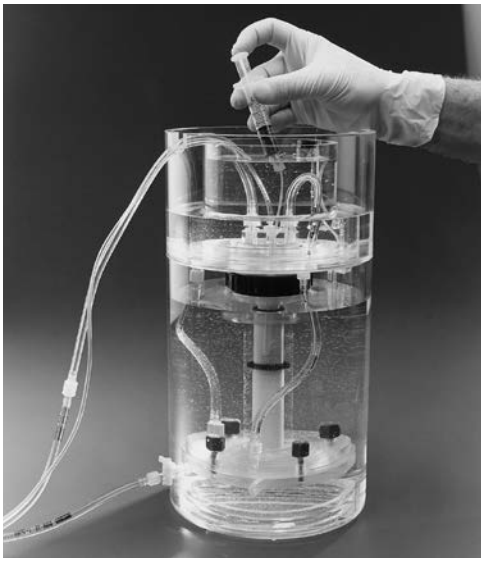


Fig. 9. Cooling pathway during electrophoresis. Heat generated in the gel during electrophoresis is drawn off by means of both the cooling core and the lower buffer chamber. Electrophoresis buffer from the lower buffer chamber is pumped through the cooling core which maintains an even cross sectional temperature within the gel.

3.7 Loading the Sample

Carefully load the sample on the surface of the gel through the sample loading guide with the sample application syringe. Layer the sample under the electrophoresis buffer above the gel. Make sure the stacking gel is not punctured with the PTFE tubing.

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 setting and begin electrophoresis.



3.8 Elution Rate, Detection, Collection, and Analysis

Elution Rate

For the highest yield of purified protein we recommend an elution buffer flow rate of 0.75–1.0 ml/min. Fractions of 2.5 ml usually provide sufficient separation. Fraction collection should begin after the ion/dye front has eluted. For preparative SDS-PAGE, Table 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.

Detection

Elution of molecules can be monitored with an ultraviolet detector and chart recorder. However, with preparative PAGE, a UV monitor does not usually provide an adequate representation of the fractionation. The elution profile on the chart recorder tracing cannot replace electrophoretic analysis of individual fractions for determining the distribution of proteins and the level of contamination in each fraction. The ion front will be seen on the UV chart recording as a high-absorbance peak or it will be in the fractions containing the sample-buffer dye. Individual peaks can sometimes be seen on a chart recording, but in many instances the sample will yield a broad, poorly defined trace registering slightly above baseline. See Figure 13 for an example of an elution profile for a starting sample containing only three protein bands.

Collection and Analysis

To determine which fractions contain the protein of interest, individual prep cell fractions must be analyzed by slab gel electrophoresis. 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 the level of contamination.

Fractions containing the purified protein can be pooled and concentrated as required for further analysis.

Section 4
Optimizing Running Conditions for Preparative SDS-PAGE

The Model 491 prep cell is designed for separating 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 the protein of interest needs to be separated from its nearest contaminant after traveling the entire length of the gel.

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

4.1 Gel pore size

4.3 Gel length

4.4 Gel tube diameter

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 Model 491 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 (%T) used 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). 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 (Rf) value between 0.55–0.6. Optimal resolution is not achieved with monomer concentrations other than the optimum value.

Rf values for specific proteins are obtained from the mini-gels that were run to optimize conditions for the Model 491 prep cell. To calculate Rf values for specific proteins use this formula:

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

The Rf value obtained from a mini gel can be used to estimate when a protein will elute from the Model 491 prep cell when the same concentration of acrylamide is used in both the mini gel and the preparative gel.

For those samples in which the molecular weight difference between the protein of interest and its nearest contaminant is ≥10%, the optimum %T can be selected from Figure 10 in the following section. For those samples in which the molecular weight difference between the protein of interest and its nearest contaminant is ≤10%, consult Section 4.3.

4.2 Optimization Procedures

A. 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. For gel length and gel tube size determination refer to Sections 4.3 and 4.4.

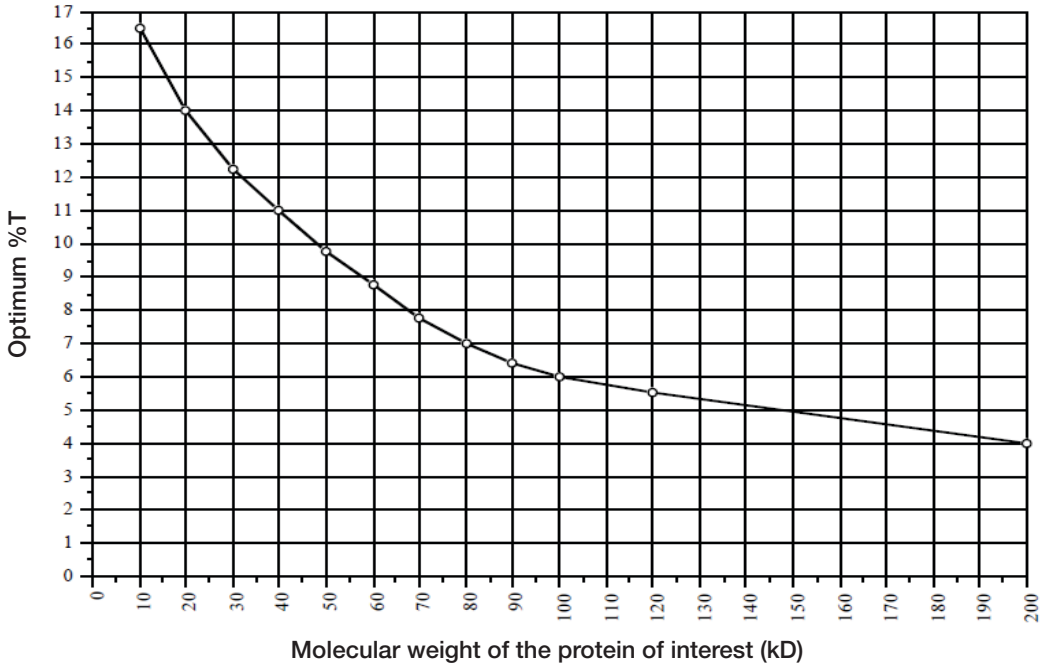


Fig. 10. Model 491 prep cell SDS-PAGE gel optimization curve. In cases where the difference in molecular weight between the protein of interest and its nearest contaminant is 10% or greater, the appropriate acrylamide concentration to purify the protein of interest from it nearest contaminant can be determined using this standard curve.

B. 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 Model 491 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 1 gives recommended ranges of monomer concentrations for various protein molecular weights. Use Figure 10 to obtain a midpoint for the range of gels to cast.

Table 1. Recommended monomer concentrations.

Size Range	%T Range
15–30 kD	6–10%
30–50 kD	9–12%
50–70 kD	7–10%
70–100 kD	5–9%
100-200 kD	4–8%

The procedure goes as follows:

1. Cast 3–4 polyacrylamide mini-slab gels in the range suggested in Table 1 (and Figure 10).
2. Load a sufficient amount of protein for detection by silver staining (~100 ng/lane). Load at least one lane with 10 µl 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, 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 protein bands of interest (protein 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. Please see Section 4.7 for examples.

4.3 Gel Length Determination for Preparative SDS-PAGE

Gel length is used to increase the resolution between proteins. However, longer gels are accompanied by an increase in band diffusion. 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 (ΔMW) is inversely related to the gel length. Small size differences require longer gels to produce the best resolution. 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 2 to select the gel length for optimizing resolution and recovery of the protein of interest.

4.4 Gel Tube Size Selection

Both the protein load and the molecular weight difference between the protein of interest and its nearest contaminant must be considered when selecting the size of the gel tube. Two different gel tubes are provided: a 28 mm (internal diameter) gel tube which forms a gel with a 3.6 cm^2 gel upper surface and a 37 mm (internal diameter) gel tube with an 8.2 cm^2 gel surface area. Resolution between proteins with very small molecular weight differences will improve approximately 1.5-fold for identical protein loads when the 37 mm column is used instead of the 28 mm. This increase in resolution is accompanied by a higher dilution factor due to the larger volume elution chamber.

The larger gel surface of the 37 mm tube also allows for greater sample loads than does the 28 mm tube. Tighter bands can be maintained by distributing the protein over a larger area.

Refer to Table 2 to determine the correct tube size and gel length for your purification scheme.

The following guidelines are established for optimizing resolution and recovery of the protein of interest with the least amount of dilution. The recommended protein loads in Table 2 refer to the amount of 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 2. Small gel tube (28 mm ID).

ΔMW (a)	Protein Load (b)	Monomer Volume (c)	Gel Length
>15%	<1 mg	20 ml	4–6 cm
>15%	1–2 mg	20–40 ml	4–6 cm
>15%	>2 mg	proceed to table 3	–
10–15%	<0.75 mg	20 ml	4–6 cm
10–15%	0.75–1.5 mg	20–40 ml	6–10 cm
10–15%	>1.5 mg	proceed to table 3	–
2–10%	<0.5 mg	20 ml	4–6 cm
2–10%	0.5–1 mg	20–40 ml	6–10 cm
2–10%	>1 mg	proceed to table 3	–

Table 3. Large gel tube (37 mm ID).

ΔMW (a)	Protein Load (b)	Monomer Volume (c)	Gel Length
>15%	<2 mg	50 ml	6 cm
>15%	2–4 mg	50–100 ml	6–10 cm
>15%	>4 mg	100 ml	10 cm (d)
10–15%	<1.5 mg	50 ml	6 cm
10–15%	1.5–3 mg	50–100 ml	6–8 cm
10–15%	>3 mg	80 ml	10 cm (d)
2–10%	1 mg	50 ml	6 cm
2–10%	1–2 mg	50–100 ml	6–10 cm
2–10%	>2mg	110 ml	10 cm (d)

(a) ΔMW 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 and is independent of the total protein load.

(c) The monomer volume recommended is based on using the optimum %T as established from analytical gels.

(d) Loads greater than the recommended amounts may lead to corresponding loss of resolution.

4.5 Running Conditions

We recommend running SDS-PAGE gels in the Model 491 prep cell using 12 W constant power. Values for voltage and current should correspond to the settings listed in Table 4. Different running conditions apply for native-PAGE (Section 9).

Table 4. Running conditions for preparative SDS-PAGE.

12 W Constant Power	
Small gel tube (28 mm ID)	40–50 mA/240–300 V
Large gel tube (37 mm ID)	50–60 mA/200–400 V

4.6 Elution Times for Proteins

Elution times for proteins run through SDS-PAGE gels at 12 W constant power are estimated in Table 5. When SDS-PAGE is carried out at the optimum %T, the relative mobility (Rf) 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 times for preparative SDS-PAGE are affected by gel length and gel tube size only and can be predicted. The elution times for both the ion/dye front and the purified protein are provided in Table 5.

Table 5. Elution times at optimal %T.

Gel Length ,cm	Elution Time for Proteins (Rf~0.55), hr	Elution Time for Ion Front (Rf=1), hr
28 mm gel tube		
5	3.5	2.0
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
37 mm gel tube		
5	6.0	3.0
6	6.75	3.5
7	7.75	4.0
8	8.5	4.5
9	10.0	5.25
10	11.0	5.5

*The elution times are approximate and will vary somewhat depending on the exact run conditions.

4.7 Examples of the SDS-PAGE Optimization Procedure

The following two examples demonstrate optimization protocols for separating two closely spaced proteins on the Model 491 prep cell. In each example, a series of mini-slab gels were run to determine the best acrylamide concentration to use in the preparative gel.

Example 1:
Purification of the Subunits of Phycocyanin

Phycocyanin, purified by ion exchange chromatography, contains two naturally colored blue protein subunits of ~18.5 kD and ~21 kD and a third, uncolored, 23 kD subunit. In this case, since the subunits of phycocyanin are well separated in size, it is possible to purify all three in a single run.

Five analytical mini slab gels, 12%, 14%, 16%, 18%, and 20%T/2.67%C, were cast to determine the %T that would provide maximal separation of the two largest subunits. The migration of the blue protein bands could be monitored directly without the use of prestained SDS-PAGE standards. Gels were run under standard SDS-PAGE conditions until the faster running protein (18.5 kD) was 5 mm from the bottom of the gels. The gels were silver stained and dried, and the distances between the two blue phycocyanin bands were measured and plotted versus %T. An optimal %T of approximately 14% for resolving these two proteins was indicated by the breakpoint, or cusp, of this curve. Refer to Figure 11.

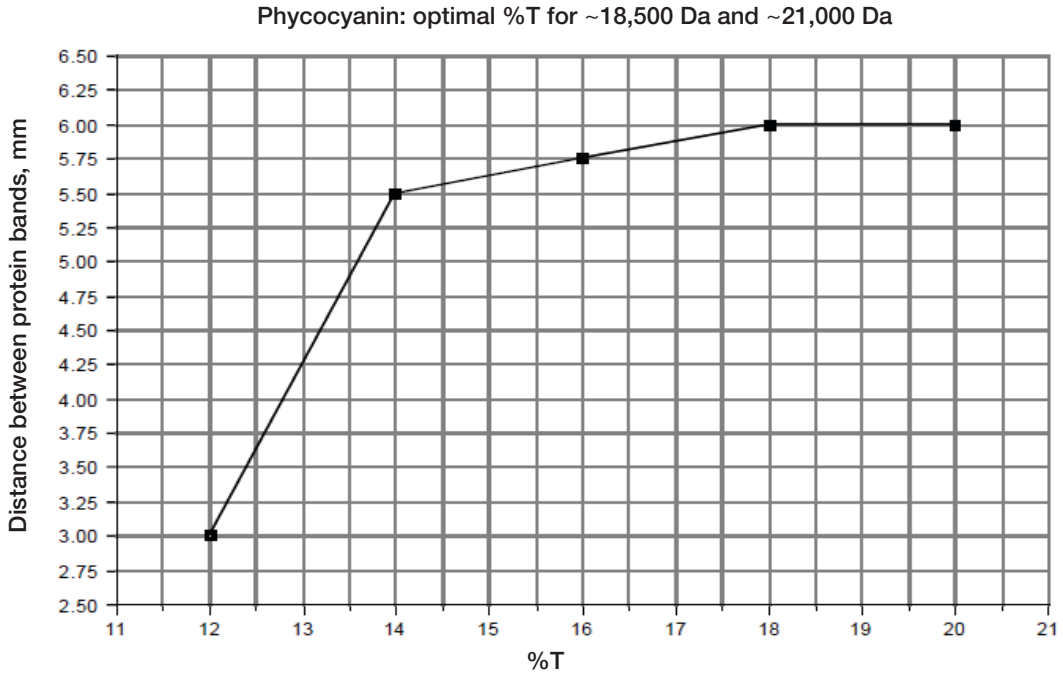


Fig. 11. Analytical SDS-PAGE for determining optimum %T for purification of 18.5 kD and 21 kD phycocyanin protein subunit bands. The break point of the curve is considered the monomer concentration for optimal separation. In this case a 14% monomer concentration is indicated.

To confirm the procedure, the Model 491 prep cell was run using the same %T range as the analytical gels. Preparative run conditions are shown in Table 6.

Table 6. Preparative run conditions.

Gel Composition	12%–17% T/2.67% bis
Gel Height	5.5 cm
Gel Size	25 mm ID
Sample Load	1 mg total protein
Running Conditions	40 mA constant current (~250–350 V)
Running Time	~5 hr

For each run the resolution (R = peak separation/average peak width) was determined. The results of the preparative runs shown in Figure 12 show how the optimal %T produces maximum resolution. The same optimum %T of 14% as with the mini gel method was found. This plot demonstrates loss of resolution at non-optimal gel concentrations. Reconstruction experiments with addition of irrelevant proteins showed that regardless of the complexity of the sample of which phycocyanin was a part, an optimal %T of 14% best resolved its two largest protein subunits.

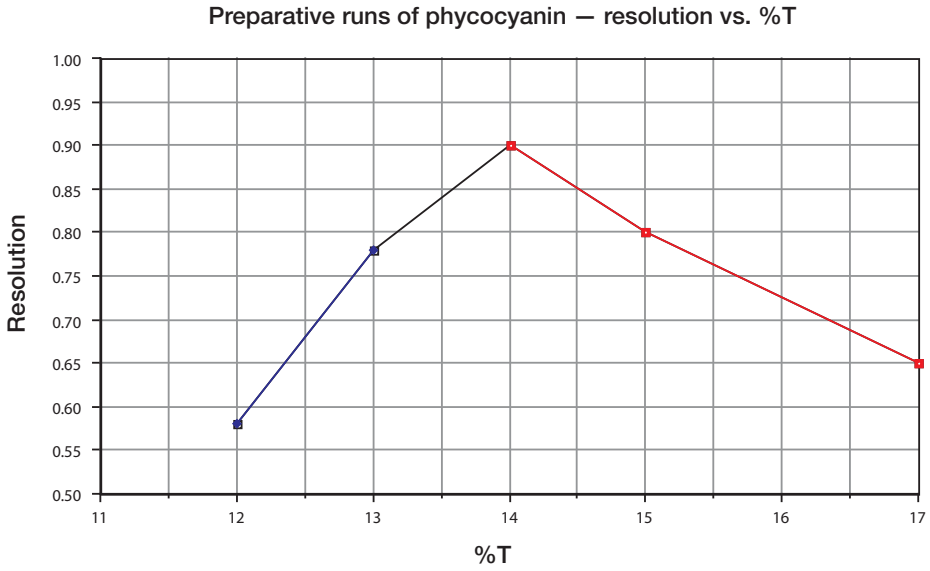


Fig. 12. Confirmation of optimum %T for preparative SDS-PAGE to separate two phycocyanin protein subunits (~18.5 kD and ~21 kD). As in chromatography, resolution of two adjacent components is defined as the ratio of peak separation to the mean peak width assuming the peaks are Gaussian in shape (i.e., R=peak separation/average peak width).

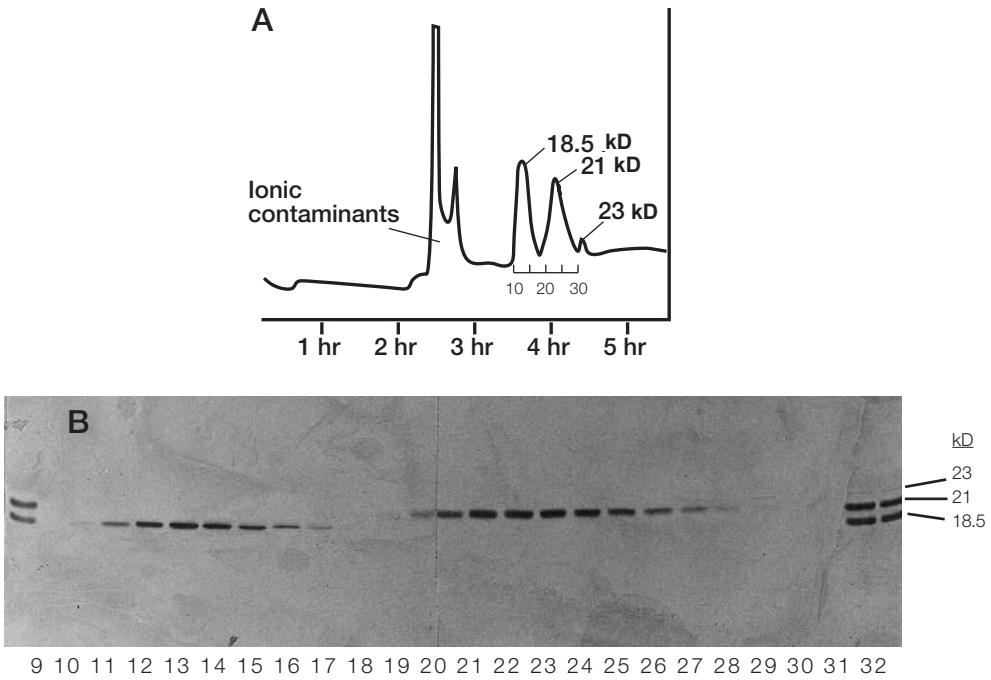


Fig. 13. Elution profile and SDS-PAGE analysis. A, Elution profile. Chromatographically purified phycocyanin was separated into three subunits (18.5 kD, 21 kD, and 23 kD) by preparative SDS-PAGE in the Model 491 prep cell. **B**, Aliquots from the Model 491 prep cell fractions 9-32 containing the separated protein subunits of phycocyanin were analyzed by SDS-PAGE gels (14%T) and silver stained. Starting material was run in the extreme left and right lanes. The low molecular weight subunit (18.5 kD) eluted in the Model 491 prep cell fractions 9–17, the mid-sized subunit (21 kD) in fractions 18–29 and the largest subunit (23 kD) eluted in fractions 29–32.

Example 2:
Purification of a 98 kD Subunit from a 96 kD Subunit of Keyhole Limpet Hemocyanin

Keyhole limpet hemocyanin analyzed on SDS-PAGE gels shows several bands covering a broad range of molecular weights. See Figure 15A. Among these are two proteins of approximately 96 kD and 98 kD representing a difference in molecular weight of approximately 2%. To determine the optimal %T for resolving these two proteins, four analytical gels of 5%, 6%, 8%, and 10%T/2.67%C were cast. Hemocyanin was electrophoresed on each gel along with two lanes of prestained SDS-PAGE standards. The prestained standards were used to monitor the position of the proteins of interest. Runs were continued until the prestained BSA marker (~80 kD) of the prestained standards had just run off the gel. The gels were silver stained and dried, and the distance between the two hemocyanin bands (96 kD and 98 kD) was measured and plotted versus %T. An optimal %T of approximately 7% is indicated for resolving these two proteins. See Figure 14.

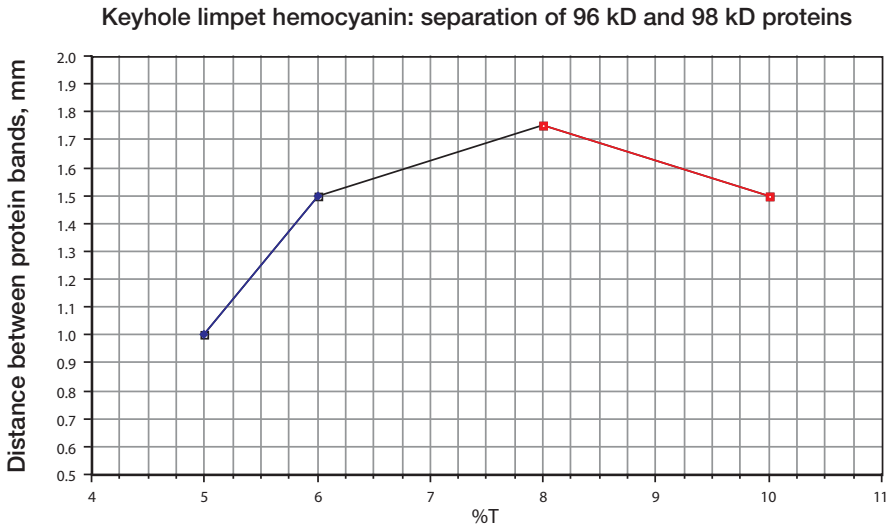


Fig. 14. Determination of optimal %T for 96 kD and 98 kD keyhole limpet hemocyanin proteins. The breakpoint of the curve is considered the monomer concentration for optimal separation. In this case a 7%T/2.67%C monomer concentration is indicated.

Based on optimal %T data obtained from analytical mini-gels, subsequent preparative run conditions were as shown in Table 7.

Table 7. Preparative run conditions based on optimal %T data obtained from analytical mini-gels.

Gel Composition	7% T/2.67% C
Gel Height	10 cm
Gel Size	28 mm
Sample Load	1 mg total protein
Running Conditions	40 mA constant current (250-350 V)
Running Time	8–10 hr

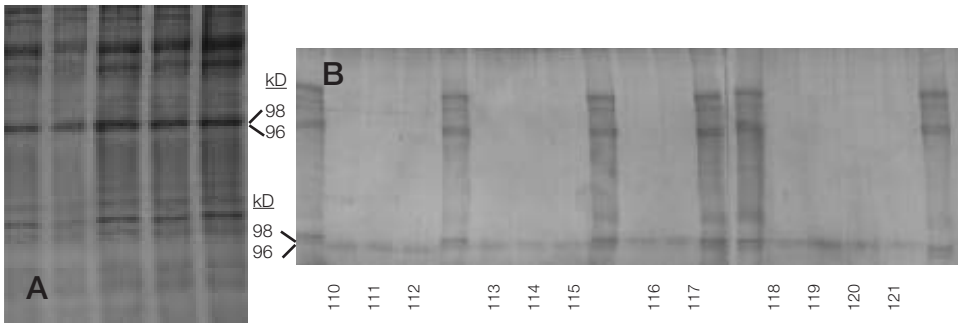


Fig. 15. Crude keyhole limpet hemocyanin (KLH) analyzed by SDS-PAGE. A, The locations of the 96 kD and the 98 kD bands are indicated. **B**, SDS-PAGE analysis of aliquots taken from Model 491 prep cell fractions. The Model 491 prep cell separated the two subunits of keyhole limpet hemocyanin into two consecutive sets of six 2.5 ml nonoverlapping fractions. Crude KLH was run in about every fourth lane. The 96 kD protein eluted in fractions 110–115. The 98 kD protein eluted in fractions 116–117. There was no overlap.

Section 5
Disassembling and Cleaning

1. Turn off the power supply, disconnect the cables from the power supply, and remove the lid. Turn off both the elution and cooling pumps.
2. Disconnect the elution buffer tubing from the cooling core. Close the valve of the cooling buffer outlet on the lower reservoir and disconnect the cooling pump tubing from the lower reservoir and the cooling core.
3. 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 and cooling buffer feedline from the elution chamber cap. Disconnect the cooling buffer line from the cooling core and unscrew the black ring nut. Remove the upper reservoir from the gel tube assembly.
4. Loosen the screws in the elution chamber cap and remove the gel tube assembly from the elution chamber base. Rinse the support frits and the elution frit with deionized water and place them in a container of buffer. 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.
5. Unlock and turn the cooling core several times to loosen it from the gel. Holding the gel tube in one hand and the neck of the cooling core in the other, carefully pull out the cooling core. The gel may be pulled up along with the cooling core. Use a soft implement to remove the gel from the gel tube. Wash the gel tube and the cooling core with Bio-Rad Cleaning Concentrate (catalog number 161-0722). A bottle brush may be used to remove residual polyacrylamide from the gel tube and cooling core. Do not scratch or otherwise abrade the interior surface of the glass tube or the glazing of the cooling core. Insure that the collar of the gel tube assembly is free of residual polyacrylamide.
6. Decant the buffer from the lower reservoir. Rinse the Model 491 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, desalting column, etc.
2. Poor resolution.	a. Sample overloaded.	a. Decrease sample load.
	b. Incorrect %T.	b. Refer to section 4.
	c. Incorrect gel length or gel tube size.	c. Check Table 2.
	d. Inadequate cooling.	d. Cooling flow rate should be 100 ml/min.
3. No detectable proteins in collected fractions.	a. Proteins too dilute and UV detection not sufficient.	a. Use silver stained SDS-PAGE gels to analyze individual prep cell fractions.
	b. Insufficient protein load.	b. Increase total protein loaded.
	c. %T too high and proteins remain in gel or diffuse to undetectable level.	c. Decrease %T. See detailed optimization procedure, section 4.2.
	d. %T too low, protein migrates with ion front.	d. Increase %T. See detailed optimization procedure in Section 4.
4. Elution buffer floods down into lower buffer chamber.	a. O-ring or dialysis membrane in elu-tion chamber missing or damaged.	a. Check to see if O-ring and dialysis membrane are in place and not damaged.
5. Air pockets between gel and gel tube.	a. Mechanical stress.	a. Reduce handling of the gel during polymerization.
	b. It is normal for a gel to pull slightly away from the wall.	b. Refractive phenomena often accentuate the appearance of these regions. They will not affect protein resolution.
	c. Polymerization too fast.	c. Check catalyst concentration.
	d. Heat of polymerization not dissipated.	d. Cool gel during polymerization.
6. Running conditions outside recommended range.	a. Buffer concentration or pH are incorrect.	a. Make fresh buffer.

Section 7
Preparation of Electrophoresis Buffers and Acrylamide Stock Solutions for SDS-PAGE

The Model 491 prep cell has three buffer reservoirs: one for the lower electrophoresis buffer (~3 liters), one for upper electrophoresis buffer (~600 milliliters) and one for the elution buffer (~900 milliliters). For SDS-PAGE all three reservoirs contain the same buffer (Laemmli buffer system). It is recommended that 6.0 liters of buffer be prepared for each preparative run. Premixed liquid acrylamide (concentrated solution) and premixed electrophoresis buffers (10x) are available. (See product information Section.)

7.1 Separating (Resolving) Gel Buffer Stock

1.5 M Tris-HCl pH 8.8
Dissolve 27.23 grams Tris base in approximately 100 ml deionized water.
Adjust to pH 8.8 with 6 N HCl.
Make to 150 ml with deionized water and store at 4 ˚C.

7.2 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 6 N HCl.
Make to 100 ml with deionized water and store at 4 ˚C.

7.3 Sample Buffer (SDS-Reducing Buffer)

Deionized water	3.8 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	0.8 ml
10% (w/v) SDS*	1.6 ml
0.5% bromophenol blue (optional)	0.4 ml
Total volume	7.6 ml

Add 50 µl beta-mercapto-ethanol to 950 µl of sample buffer prior to use. Dilute the sample at least 1:4 with sample buffer, and heat at 95˚C for 4 min.

*To make a 10% SDS solution: Dissolve 10 g of SDS in deionized water with gentle stirring and bring to 100 ml with water.

SDS reducing buffer is 0.06 M Tris-HCl, pH 6.8, 2% SDS, 5% beta-mercapto-ethanol, 10% glycerol and 0.025% bromophenol blue.

Very dilute samples can often be adequately reduced with a two-fold dilution (1:1) with sample buffer.

7.4 10x Electrode (Running) Buffer, pH 8.3

(Makes 1 L)

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 6 L of 1x electrophoresis buffer (0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3) for the prep cell, dilute 600 ml of 10x stock with 5,400 ml deionized water.

7.5 30% Acrylamide Stock Solution

Acrylamide/bis (N,N'-bis-methylene-acrylamide)
(30%T/2.67%C)

Acrylamide	146.0 g
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.

(Preweighed 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
SDS-PAGE Gel Preparation

8.1 Gel Recipes

Table 8 can be used to prepare both analytical and preparative Tris-HCl acrylamide gels. Sections 8.2–8.3 provide detailed formulas for calculating specific gel percentages and volumes. Acrylamide/bis stock solution of 30% (37.5:1) are used. The amounts listed for the components in Table 8 are based on a total volume of 10 ml. Determine the total volume needed and multiply each component by the appropriate number. As long as 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. Gel preparation reference.

(Acrylamide Monomer) %T	ddi H2O, 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	5.45	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
11	3.80	2.50	3.67
12	3.45	2.50	4.00
13	3.15	2.50	4.33
14	2.80	2.50	4.67
15	2.45	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

Table 9. Catalyst preparation reference.

	Catalyst	
	10% APS* (µl)	TEMED* (µl)
Analytical resolving gel	**50**	**5**
Stacking Gel	**50**	**10**
Preparative resolving gel	**25**	**2.5**
Stacking gel	**50**	**10**

**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.

8.2 Analytical Separating Gels

Table 10. Calculating %T (0.375 M Tris, pH 8.8).

Separating Monomer Concentration = %T = %x	
Acrylamide/bis (30% T/2.67%C Stock)	c ml
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 2 mini gels

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

c ml: Calculate the volume of 30% acrylamide/bis stock required for 10 ml of the desired total monomer concentration with the following formula:
c ml = (%x)(10 ml)/(30%) = (x)(0.333) ml

d ml: Calculate the volume of water required at the desired total monomer concentration with the following formula:
d ml = 10 ml – 2.5 ml – 50 µl – 5 µl – c ml = 7.445 ml – c 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

Table 11. 4%T/2.67%C (0.125 M Tris, pH 6.8).

Stacking Monomer Concentration =	40% T/2.67% C
Acrylamide/bis (30% T/2.67%C stock)	1.33 ml
0.5 M Tris HCl, pH 6.8	6.10 ml
Deionized water	2.5 ml
10% ammonium persulfate (fresh daily)	50 µl
TEMED	10 µl
Total monomer	10 ml (volume needed for 2 stacking gels

8.4 Preparative SDS-PAGE Separating and Stacking Gels

(The following calculations are based on the preparation of a full length separating gel of ~11 cm with a ~2.5 cm stacking gel in the 28 mm ID gel tube)

Table 12. Calculating %T.

Monomer Concentration	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
0.5 M Tris HCl, pH 8.8	10.0 ml	–
1.5 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:

c ml = (x%) (40 ml)/% acrylamide stock solution

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

d ml = 40 ml – 10 ml – 100 µl – 10 µl – c ml = 29.89 ml – c ml

The volume of the stacking gel should be one-and-one-half to twice the sample volume to be electrophoresed. Large sample volumes will require large stacking gels, which will limit the length of the resolving gel.

Section 9
A Guide to Preparative Native-PAGE

9.1 Introduction

Conventional gel electrophoresis buffer systems and media are used with the Model 491 prep cell to separate individual components from their nearest contaminants. This guide describes a method for selecting the best nondenaturing PAGE system to isolate a particular protein with the Model 491 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 and therefore 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 towards 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 towards 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 buffers 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 Model 491 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 nondenaturing gel system tried (Ornstein 1964). Detailed protocols are provided in Section 9.3 for using this system. In discontinuous systems, the buffer used in the stacking gel, resolving gel and in the electrode chambers differs in ionic concentration and pH. 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. Section 9.2 presents an alternative to using discontinuous buffer systems.

Continuous Buffer Systems

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 (Chrambach and Jovin 1984). The electrophoresis buffers described in this article span the pH range from 3–10. Protocols for using these discontinuous buffers are analagous to those listed in Section 3 for the Ornstein-Davis buffer system.

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 that can be tried (Mclellan 1982). See Section 9.4 for preparation of these continuous buffers.

For in-depth discussions of electrophoresis and the distinctions between continuous and discontinuous systems, the Hames, Andrews, and Allen references in Section 9.5.

Optimizing Conditions for Preparative Native-PAGE

Conditions for purification of native proteins with the Model 491 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 Model 491 prep cell. Figure 16 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 Model 491 prep cell.

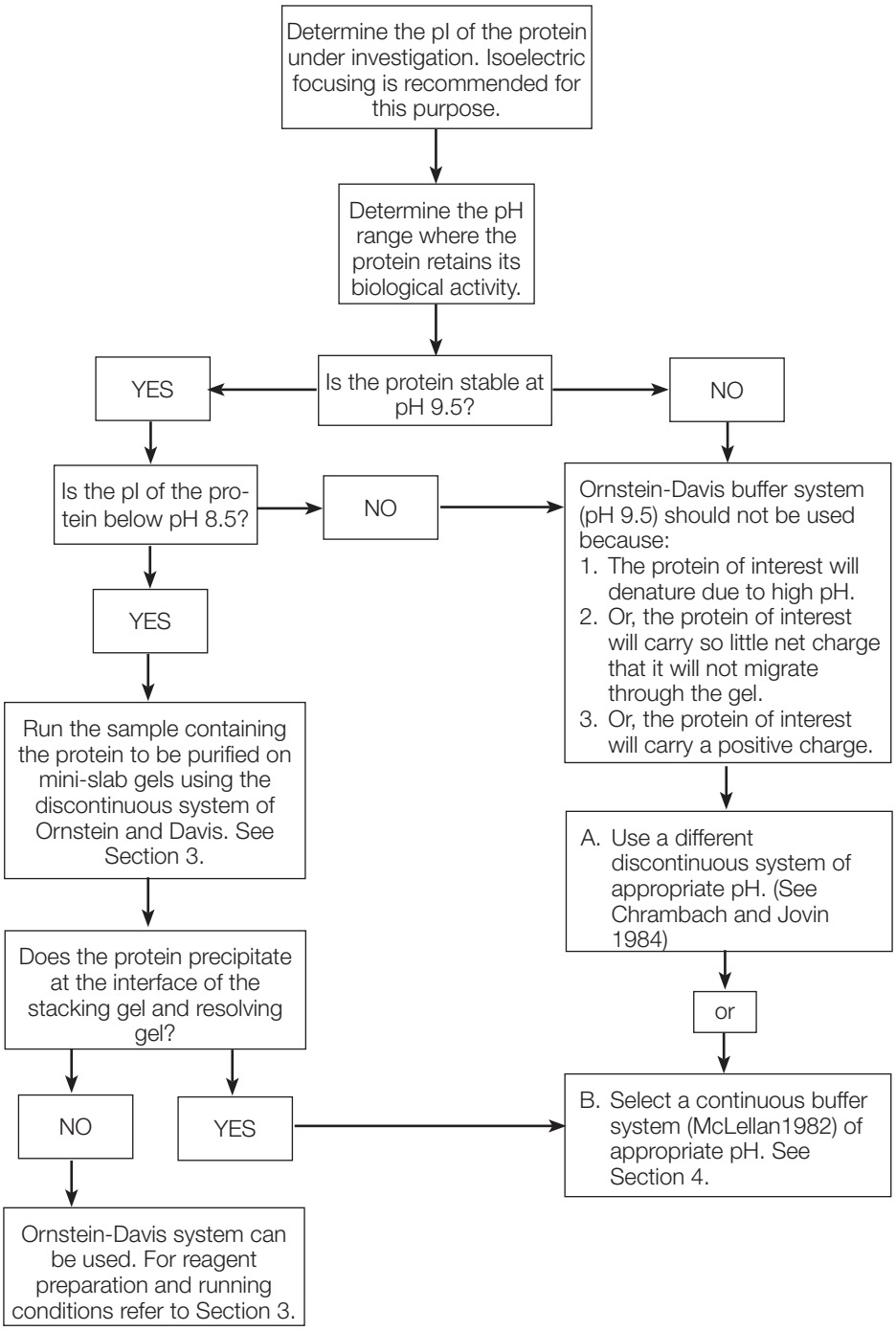


Fig. 16. Selecting a native electrophoresis buffer system.

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.

The mobilities of native proteins can be modified 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 Model 491 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 (Rf) of the protein of interest and its nearest contaminant. (To calculate Rf values see Section 3.7.) 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 Rf between these two proteins. This is the %T to use for preparative native-PAGE in the Model 491 prep cell.

Polyacrylamide Gel Recipes

A. 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.
(Preweighed Acrylamide/bis 37.5:1 mixture or 30% acrylamide/bis stock solution can be substituted.

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 to create a gel, induce polymerization, and discard the solidified gel.

Preparing Ornstein-Davis Native Electrophoresis Buffer Solutions

A. 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.

B. 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.

C. Sample Buffer (0.0625 M Tris-Cl, pH 6.8, 10% Glycerol, 0.025% Bromophenol Blue)

Deionized water - 5.8 ml
0.5 M Tris-HCl, pH 6.8 - 1.0 ml
Glycerol - 0.8 ml
0.5% Bromophenol blue (optional) - 0.4 ml
Total volume - 8.0 ml

D. 10x Electrode Running Buffer, pH 8.3 (Makes 1 L)

Tris base - 30.3 g
Glycine - 144.0 g

Dissolve and adjust to 1000 ml with deionized water. DO NOT adjust pH with acid or base.

E. To make 1x Electrode Running Buffer (6 L; 0.025 M Tris, 0.192 M Glycine, pH 8.3)

Dilute 600 ml 10x electrophoresis buffer (pH 8.3) with 5,400 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. Determine the total volume needed and multiply each component by the appropriate number.

Table 13. Reference table for the preparation of acrylamide gels (10 ml volume).

%T	Deionized H_2O, ml	Gel Buffer*, ml	Bis-acrylamide 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	4.45	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.

Table 14. Reference table for the preparation catalyst solutions.

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

****Note**: To slow the rate of polymerizaton 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.

Consult Sections 3 and 4 in this manual for an in-depth discussion about casting preparative gels, sample loading and running conditions.

Running Conditions for Discontinuous Native-PAGE Using the Prep Cell

A. Run the Model 491 prep cell at 12 W 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.

B. An elution flow rate of 0.75–1.0 ml/minute is recommended. Fractions should be collected following elution of the ion front.

C. Protein migration rates of 1.0–2.0 cm/hour correspond to approximate elution times of 3–6 hr for a 5 cm gel.

D. The migration rates of proteins run in Ornstein-Davis gels at 12 W constant power will approximate those shown in Table 15.

Table 15. Approximate migration rates of proteins run in Ornstein-Davis gels at 12 W constant power.

Rf	Migration Rate
1.0	30 mn/cm gel
0.8	40 mn/cm gel
0.6	50 mn/cm gel
0.45	60 mn/cm gel

Rf values for specific proteins are obtained from the mini-gels that were run to optimize conditions for the Model 491 prep cell. To calculate Rf values for specific proteins use this formula:

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

The Rf value obtained from a mini gel can be used to estimate when a protein will elute from the Model 491 prep cell when the same concentration of acrylamide is used in both the mini gel and the preparative gel. Rf values below 0.45 may result in excessive band broadening and loss of resolution.

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 tall 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 Table 16 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 for preparation of a 6% acrylamide gel. Electrophoresis is carried out at approximately 200 V 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 approximately 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 (McLellan 1982). Use the table below to prepare 1 liter of 5x continuous nondenaturing PAGE buffer. DO NOT adjust pH with acid or base. If the final pH is outside the the listed range discard the buffer and remake.

Table 16. Nondenaturing PAGE buffer preparation.

Buffer pH ± 0.1	Basic Component and MW	5x Solution g/L or ml/L	Acidic Component and MW	5x Solutions ml/L or g/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	MPOS 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/mL	CAPS 221.3 mw	22.13 g/L

To make 1 L of 1x electrophoresis buffer, dilute 200 ml of 5x buffer with 800 ml deionized water. The final concentrations of buffer should be as shown in Table 17.

Table 17. Final concentrations.

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 nondenaturing PAGE systems use relatively large pore size gels. Generally 4–6 cm long gels are sufficient for optimum resolution in the prep cell.

For 10 ml acrylamide monomer solution

Table 18. Acrylamide monomer solution per 10ml of gel volume.

%T	Deionized H_2O, ml	Continuous Buffer*, ml	Acrylamide/bis solution 30% stock (37.5:1), ml
4	6.65	2.00	1.33
5	6.30	2.00	1.67
6	5.85	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. To cast a preparative gel refer to Sections 3 and 4 in this manual.

Table 19. Catalyst quantity recommendation by type of gel.

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

****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 Model 491 Prep Cell

For continuous buffer systems, run the gels at 5 W constant power (approximately 10 mA and 500 volts). Cooling the lower buffer and (or) running the Model 491 prep cell in the cold room may help to maintain the biological activity of some proteins. Refer to Section 3.6 in this manual for details about cooling the apparatus.

The elution buffer flow rate for the Model 491 prep cell should be set to 0.75–1.0 ml/min. 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 prep cell. Analysis by SDS-PAGE can be used to confirm the resolution and purity.

For a complete discussion regarding operating the Model 491 prep cell and details about sample collection and analysis, refer to Sections 3 and 4.

9.5 References

Allen RC et al. (1984). Gel Electrophoresis and Isoelectric Focusing of Proteins: Selected Techniques. (New York: de Gruyter).

Andrews AT (1986). Electrophoresis: Theory, Techniques and Biochemical and Clinical Applications (Oxford: Clarendon Press).

Chrambach A and Jovin T (1983). Selected buffer systems for moving boundary electrophoresis on gels at various pH values, presented in a simplified manner. Electrophoresis 4, 190–204.

Hames BD (1990). Gel Electrophoresis of Proteins: A Practical Approach. D. Rickwood and BD Hames ed. (New York: Oxford University Press).

McLellan T (1982). Electrophoresis buffers for polyacrylamide gels at various ph. Anal Biochem 126, 94-99.

Ornstein L (1964). Disc electrophoresis. I. Background and theory. Ann N Y Acad Sci 121, 321-349.

Section 10
Ordering Information

Catalog Number	Product Description
170-2925	**Model 491 Prep Cell,** without buffer recirculation pump
170-2926	**Model 491 Prep Cell**, with buffer recirculation pump (100/120VAC)
170-2927	**Model 491 Prep Cel**l, with buffer recirculation pump (220/240VAC)
170-2929	**Buffer Recirculation Pump**, 120/100 VAC
170-2930	**Buffer Recirculation Pump**, 220/240 VAC
	Replacement Accessories
170-2932	**Small Gel Tube Assembly**, 28 mm ID
170-2933	**Large Gel Tube Assembly**, 37 mm ID
170-2934	**Cooling Finger Assembly**, includes feedline connectors
170-2935	**Buffer Circulation Tubing Kit**, includes stop cock with tubing and connectors, elution buffer circulation lines and connectors (3), and electrophoresis/cooling buffer circulation lines and connectors (3).
170-2936	**O-ring Kit, upper and lower**, 2
170-2937	**Dialysis Membranes**, 5
170-2938	**Frit Kit**, includes support frit and elution frit
170-2939	**Sample Application/Overlay Buffer Kit**, includes sample loading guide and syringe with PTFE tubing.
170-2940	**Thumb Screws**, 4
170-2941	**Elution Manifold Base**
170-2942	**Lower Buffer Chamber**
170-2943	**Upper Buffer Chamber**
170-2944	**Casting Stand**
170-2969	**Lid and Safety Cable Duplex**
	Auxiliary Instruments
170-2950	**Rotofor® Preparative IEF Cell**, 100/120 VAC
170-2951	**Rotofor Preparative IEF Cell**, 220/240 VAC
165-5056	**PowerPac HV Power Supply**, 100–120/220–240 V
164-5070	**PowerPac Universal Power Supply**, 100-120/220–240 V
731-8101	**Econo System**, complete, with Model 1325 Chart Recorder, 100/120 VAC
731-8100	**Econo System**, without Chart Recorder, 100/120 VAC
731-8102	**Econo System**, without Chart Recorder, 220/240 VAC
731-8140	**Econo-Pump**, Model EP-1, 100/120 VAC
731-8142	**Econo-Pump**, Model EP-1, 220/240 VAC
731-8160	**Econo UV Monitor**, Model EM-1, 100/120 VAC
731-8162	**Econo UV Monitor**, Model EM-1, 220/240 VAC
731-8122	**Fraction Collector**, Model 2110, 100/120VAC
731-8120	**Fraction Collector,** Model 2110, 220/240VAC
731-8190	**Chart Recorder**, Model 1325, 100/120 VAC
731-8192	**Chart Recorder**, Model 1325, 220/240 VAC

Electrophoresis Chemicals

Catalog Number	Product Description	Quantity/ Package
Premixed Electrophoresis Buffers		
161-0732	**10x Tris/Glycine/SDS Buffer**	1 L
161-0755	**10x Tris/Glycine/SDS Buffer 6 x**	1 L
Premixed Acrylamide/bis		
161-0122	**37.5:1 mixture**, (2.67% C)	30 g
161-0125	**37.5:1 mixture**, (2.67% C)	150 g
30% Acrylamide/bis Solutions		
161-0158	**37.5:1 mixture**, (2.67% C)	500 ml
161-0159	**37.5:1 mixture**, (2.67% C)	2 x 500 ml
161-0100	**Acrylamide**, 99.9%	100 g
161-0101	**Acrylamide**, 99.9%	500 g
161-0107	**Acrylamide**, 99.9%	1 kg
161-0103	**Acrylamide**, 99.9%	2 kg
161-0122	**Preweighed Acrylamide/bis**, 37.5:1 mixture	30 g
161-0125	**Preweighed Acrylamide/bis**, 37.5:1 mixture	150 g
161-0200	**Bis (N,N'-Methylene-bis acrylamide)**	5 g
161-0201	**Bis (N,N'-Methylene-bis acrylamide)**	50 g
161-0716	**Tris**	500 g
161-0719	**Tris**	1 kg
161-0717	**Glycine**	250 g
161-0718	**Glycine**	1 kg
161-0300	**SDS (Sodium dodecylsulfate)**	25 g
161-0301	**SDS (Sodium dodecylsulfate)**	**100 g**
161-0302	**SDS (Sodium dodecylsulfate)**	1 kg
161-0700	**Ammonium Persulfate**	10 g
161-0610	**Dithiothreitol**	1 g
161-0611	**Dithiothreitol**	5 g
161-0710	**2-mercaptoethanol**	25 ml
161-0800	**TEMED**	5 ml
161-0801	**TEMED**	50 ml
162-0100	**Agarose**, Standard Low -m_r	100 g
162-0102	**Agarose**, Standard Low -mr	500 g
161-0363	**Precision Plus Protein Unstained Standards**,	1 ml
161-0374	**Precision Plus Dual Color Standards**,	500 ul
161-0310	**IEF Standards** (pH 4 to 10)	
161-0443	**Silver Stain Kit**, includes 1 bottle oxidizer concentrate, 1 bottle silver reagent concentrate; and 4 bottles developer enough to stain approximately 24 gels.	1 kit
161-0400	**Coomassie Blue R-250**	10 g
161-0404	**Bromophenol Blue**	10 g
161-0407	**Triton X-100**	500 ml
161-0460	**CHAPS**	1 g
161-0465	**CHAPSO**	1 g
161-0730	**Urea**	250 g
161-0731	**Urea**	1 kg
142-6424	**AG 501-X8 Ion Exchange Resin**	1 lb

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