

# **Model 422 Electro-Eluter**

## **Instruction Manual**

**Catalog Numbers  
165-2976 and 165-2977**

For Technical Service  
Call Your Local Bio-Rad Office or  
in the U.S. Call **1-800-4BIORAD**  
(1-800-424-6723)

## Note

To insure best performance from the Model 422 Electro-Eluter, become fully acquainted with these operating instructions before using the cell to separate samples. Bio-Rad recommends that you first read these instructions carefully. Then assemble and disassemble the cell completely without casting a gel. After these preliminary steps, you should be ready to cast and run a gel.

Bio-Rad also recommends that all Model 422 Electro-Eluter 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** \_\_\_\_\_

**Warranty Period** \_\_\_\_\_

**Serial No.** \_\_\_\_\_

**Invoice No.** \_\_\_\_\_

**Purchase Order No.** \_\_\_\_\_

## Warranty

Bio-Rad Laboratories warrants the Model 422 Electro-Eluter against defects in materials and workmanship for 1 year. 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. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of fittings or other spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by accident or misuse.
5. Damage caused by disaster.
6. Corrosion due to use of improper solvent or sample.

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

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## Section 1 Introduction

Elution of proteins and nucleic acids from gel slices is often a difficult and unreliable procedure. The Model 422 Electro-Eluter solves many of the problems of conventional eluters by providing easy assembly and eliminating sample loss due to leakage. The Model 422 Electro-Eluter consistently yields between 70% and 100% of the starting material, depending on the type of elution. The samples are collected in 400-600  $\mu$ l volumes. The small volume of eluted material makes subsequent manipulations easy. The Model 422 Electro-Eluter can run up to six samples at once, with no increase in running time or decrease in yield.

### 1.1 Specifications

#### Construction

Electrode module	Precision machined acrylic
Buffer chamber and lid	Molded polycarbonate
Glass tubes	Borosilicate glass
Frits	Porous polypropylene
Silicone adaptors	Molded silicone
Grommets and stoppers	Molded silicone
Membrane caps	Molded polyethylene with dialysis membrane

**Overall size** 16 cm (L) x 12 cm (W) x 18 cm (H)

**Elution capacity** 1 to 6 samples

**Glass tube dimensions** 1 cm ID x 6 cm long

**Collection volume** 400-600  $\mu$ l

**Buffer volume** 700 ml

**Shipping weight** 1.1 kg

**Chemical compatibility** The Model 422 Electro-Eluter is not compatible with chlorinated hydrocarbons (e.g. chloroform), aromatic hydrocarbons (e.g. toluene, benzene), or acetone. **Use of organic solvents voids all warranties.**

## Section 2 Equipment and Reagents

### 2.1 Equipment

<b>Catalog Number</b>	<b>Product Description</b>
165-2976	<b>Model 422 Electro-Eluter</b> , includes electro-eluter module, 12 Membrane Caps with a molecular weight cut off (MWCO) of 12,000-15,000 daltons, 6 Glass Tubes with Frits, 6 Silicone Adaptors, 6 Grommets and Stoppers, buffer tank with lid and cables, and instruction manual
165-2977	<b>Model 422 Electro-Eluter Module</b> , same as 165-2976 without buffer tank and lid
<b>Accessories</b>	
165-2985	<b>Clear Membrane Caps (12)</b> , MWCO of 12,000-15,000 daltons
165-2986	<b>Green Membrane Caps (12)</b> , MWCO of 3,500 daltons
165-2978	<b>Glass Tubes (6)</b>
165-2981	<b>Silicone Adaptors (6)</b>
165-1988	<b>Grommets and Stoppers (8)</b>
165-2987	<b>Frits (12)</b>
<b>Related Accessories</b>	
161-0719	<b>Tris</b> , 1 kg
161-0718	<b>Glycine</b> , 1 kg
161-0400	<b>Coomassie Blue R-250</b> , 10 g
165-4710	<b>Model 1000/500 Power Supply</b> , 120 V, 50/60 Hz
165-4711	<b>Model 1000/500 Power Supply</b> , 220 V, 50/60 Hz

### 2.2 Modular Mini Electrophoresis System

165-2940	<b>Mini-PROTEAN® II Cell</b> , includes inner cooling core with gaskets, lower buffer chamber, lid with power cables, Glass Plates (3 sets), clamp assemblies (2), 10-well, 0.75 mm thick combs (2), casting stand with gaskets, leveling bubble, and instruction manual
170-3930	<b>Mini Trans-Blot® Electrophoretic Transfer Cell</b> , includes 2 gel holders, electrode assembly, fiber pads, Bio-Ice® cooling unit, and instruction manual
170-3935	<b>Mini Trans-Blot Module</b> , for use with the Mini-PROTEAN II cell, same as 170-6530 without buffer tank and lid
165-2960	<b>Mini-PROTEAN II 2-D Cell</b> , includes tube adaptor, sample reservoirs and stoppers, sample reservoir/capillary tube connectors, Capillary Tubes with Casting Tube, Tube Gel Ejector, Mini-PROTEAN II slab cell with inner cooling core and gaskets, lower buffer chamber, lid with power cables, 2-D combs with 1 standard well (2), 1.0 mm thick spacers (4), Casting Stand with Gaskets, leveling bubble, and 2-D instruction manual.
165-2965	<b>Mini Tube Cell Module</b> , includes tube adaptor, sample reservoirs and stoppers, sample reservoir/capillary tube connectors, Capillary Tubes with Casting Tube, Tube Gel Ejector, and instructions.

## Section 3 Safety Instructions



Power to the Model 422 Electro-Eluter is to be supplied by an external DC voltage power supply. **This power supply must be ground isolated in such a way that the DC voltage output floats with respect to ground.** All of Bio-Rad's power supplies meet this important safety requirement. Regardless of which power supply is used, the maximum specified operating parameters for the cell are:

<b>600 VDC</b> 	maximum voltage limit
<b>24 Watts</b>	maximum power limit
<b>50°C</b>	maximum ambient temperature limit



Current to the cell, provided from the external power supply, enters the unit through the lid assembly, providing a safety interlock to the user. Current to the cell is broken when the lid is removed. **Do not attempt to circumvent this safety interlock, and always turn the power supply off before removing the lid, or when working with the cell in any way.**

Since hazardous voltages may be present in the electro-eluter, it is important that certain precautions are observed for the safety of the operator:

1. Always turn off the power supply before removing the safety cover. Do not use the safety cover as an on-off switch.
2. Never attempt to operate the cell without the safety cover.
3. Do not attempt to move the cell with the power on.
4. Do not leave an operating cell unattended for long periods of time.
5. Do not overfill the upper or lower buffer reservoirs. Refer to Section 4 for proper buffer levels.
6. If the cell appears to be drawing excessive current, turn off the power supply and check the entire system (for example, buffer chamber leaks, overfilled buffer chamber, buffer too conductive, etc.).

**Note:** Please become familiar with these precautions, both for safe operation of this equipment and to prevent loss of valuable experimental results.

### **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.

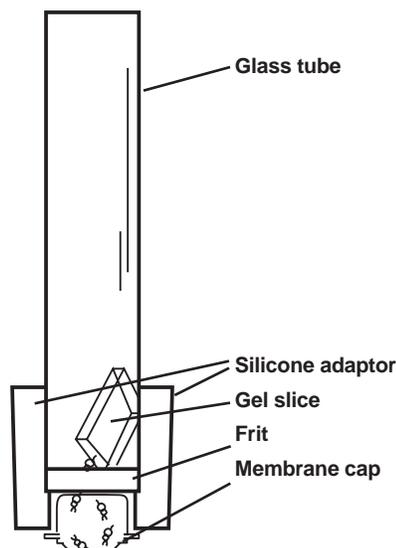
\*IEC1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

## Section 4

### Model 422 Electro-Eluter Assembly

1. Soak the Membrane Caps at 60 °C in elution buffer (see Section 9 for buffer recipes) for at least 1 hour prior to use. Membrane caps may be soaked for longer than 1 hour. Wear gloves when handling the membrane caps to prevent the dialysis membrane from becoming contaminated. Membrane caps may be reused. If they are to be reused they must be stored in elution buffer containing 0.05% sodium azide ( $\text{NaN}_3$ ) in the refrigerator. They do not need to be reheated or resoaked when reused.
2. Take the appropriate number of Glass Tubes and place one Frit in the bottom of each tube. Set the tube on a clean paper towel and, if necessary, push on the Frit from inside the Glass Tube to insure that the Frit is flush with the bottom of the glass tube. Next, push the Glass Tube and Frit into the electro-eluter module. It is easiest to do this by wetting the grommet with elution buffer and sliding the Glass Tube in place. Make sure the Glass Tube is even with the top of the grommet and the Frit is flush with the bottom of the Glass Tube. Fill any empty grommet holes with stoppers.
3. Wear gloves when handling the Membrane Caps to prevent contamination of the dialysis membrane. Place one pre-wetted membrane cap in the bottom of each Silicone Adaptor. Fill the adaptor with elution buffer. Pipet the buffer up and down to remove any air bubbles around the dialysis membrane.
4. Slide the Silicone Adaptor with the membrane cap onto the bottom of the Glass Tube with Frit. Some tiny air bubbles may come out of the Frit. Pull the Silicone Adaptor partially on and off a few times to make sure all the air bubbles are expelled. All air bubbles must be removed from the Frit for best elution yields. See Figure 1.

**Note:** The glass tubes for the Model 422 Electro-Eluter have one frosted end. The white silicone adaptor must be placed on the frosted end, or it may slip off during electro-elution.



**Fig. 1. Side view of Model 422 Electro-Eluter assembly.** The vertical glass tube is filled with elution buffer. The negative electrode is at the top. The positive electrode is below the membrane cap. Macromolecules are carried by the electrical current out of the gel slice, through the Frit and into the membrane cap. The molecules are retained by a dialysis membrane which is molded into the cap.

5. Fill each tube with elution buffer and place the gel slice in the tube. To increase sample recovery, several bands may be excised from the gel and minced, so long as the height of the gel within the glass tube is approximately one centimeter or less. If the glass tube is filled higher than 1 cm, elution times may increase.
6. Place the entire module into the buffer chamber. Fill the lower buffer chamber with ~600 ml of elution buffer. The level of the lower buffer must be above the top of the silicone adaptors or bubbles may form on the bottom of the dialysis membrane. Fill the upper buffer chamber with ~100 ml of elution buffer. To prevent electrical shorts the banana plugs must be dry.
7. Place a stir bar in the bottom buffer tank. Vigorous stirring during the run will prevent bubbles from sticking to the bottom of the dialysis membrane.
8. Attach the lid with cables, orienting the red to red (anode) and black to black (cathode).
9. Elution is done at 8-10 mA/glass tube. See Sections 6 and 8 for specific conditions for eluting protein and DNA. See Section 9 for elution buffer recipes.

## Section 5

### Protein Visualization in SDS-Polyacrylamide Gels

After the protein has been separated by gel electrophoresis, it is necessary to visualize the band of interest. If the protein can be fixed, stained, and eluted see Section 6.1. Unstained proteins can be isolated using one of the methods in Section 5.

#### 5.1 Visualizing the Protein Using Coomassie Blue R-250

Using this method, a reference lane containing the same sample as the one being eluted is run on the gel and stained to identify the band of interest. Prior to electrophoresis, the reference lane and the lane that will be eluted are treated exactly the same. The sample is prepared as one large batch and equal amounts are loaded on the elution lane and the reference lane.

1. The reference lane should be run next to the elution lane. It should not be run on the end lane because edge effects may distort the banding pattern. The gel is run at the optimal conditions for the band of interest.
2. At the end of the run, cut a corner off the gel to maintain the correct orientation. Keep the gel moist in a small amount of running buffer.
3. The reference lane is cut off, fixed, and stained with 10% acetic acid, 40% methanol, 0.1% Coomassie R-250 for 1 hour. It is destained using 10% acetic acid, 40% methanol for 2 hours, or until the band of interest can be identified. It is not necessary to have a completely clear background. The proteins will not diffuse in the 2-3 hours needed to do the staining. Line up the stained gel lane with the unstained gel, making sure the corner that was cut off is in the correct orientation. Cut out the appropriate area of the unstained gel along with some extra gel above and below the band. The staining process may cause the gel to shrink, so a larger area must be cut to insure that all of the protein is obtained.

Bands to be cut out and eluted may be spaced very close to other potentially “contaminating” bands. Shrinking of the reference lane may make it difficult to accurately determine the corresponding area to cut out from an unstained, unshrunk lane. In such a case, the unstained lane(s) can be shrunk in 10% acetic acid (without methanol and Coomassie R-250). In this way, the reduced size of the lane containing the band(s) to be eluted will correspond to the stained gel.

## 5.2 Visualizing Proteins Using Copper Chloride

An alternative method to Coomassie R-250 is the use of the new copper chloride non fixative-negative stain (Bio-Rad Copper Stain, catalog number 161-0471). This rapid, non-precipitating staining procedure surpasses Coomassie Blue in sensitivity. Following a single 5 minute incubation in  $\text{CuCl}_2$ , proteins can be quantitatively eluted from gel slices at any time. When the gel slice is obtained, proceed with the elution. See Section 6. [Copper Chloride: A Five Minute Protein Stain for Sodium Dodecyl Sulfate-Polyacrylamide Gels, *Anal. Biochem.*, **166**, 308-312 (1987).]

## 5.3 Visualizing Proteins Using Autoradiography

In this method, radioactively labeled proteins are used as the reference. An aliquot of the sample is labeled and equal amounts of labeled and unlabeled protein are loaded on separate lanes of an SDS-polyacrylamide gel. Using the labeled protein lane as a reference, the unlabeled protein of interest can be identified.

1. Load the labeled and unlabeled protein next to each other on the gel. Run the gel at the optimal conditions for the band of interest. Both lanes should be run in the middle of the gel because the end lanes may have edge effects that distort the banding pattern. Do not allow the dye front to run off the gel.
2. After the run, cut a corner off the gel to maintain correct orientation.
3. Cut the reference lane off and place it on filter paper. Using radioactive ink (ink with some extra isotope in it), label the top and bottom of the filter paper with the reference lane. Dry the reference lane. Keep the rest of the gel moist by wrapping it in plastic wrap.
4. Place the dried gel in a cassette with X-ray film and expose overnight at  $-70\text{ }^\circ\text{C}$ . After the film has been developed, align the film and the dried lane from the gel using the marks made with the radioactive ink. Using a lab marker, draw a line on the dried reference lane indicating where the band is on the autoradiogram.
5. Put the rest of the gel on a glass plate. Align the gel using the cut-off corner. Put the glass plate with the gel on top of the dried reference lane. Line up the top and bottom of the dried lane and the gel. Using the mark made on the dried lane, cut out the appropriate area of the gel. The dye front can be used to identify the lanes. Cut liberally to insure that all the protein is excised. Protein diffusion should not be a problem if the band is excised the next day.
6. After the gel slice has been cut out, proceed with the elution as described in Section 6.

## Section 6 Protein Elution

### 6.1 Elution from SDS-PAGE Gels

1. Soak the membrane caps in protein elution buffer for at least 1 hour at  $60\text{ }^\circ\text{C}$ . See Section 9 for buffer recipes. Membrane caps may be soaked for longer than 1 hour. Wear gloves when handling the Membrane Caps to prevent the dialysis membrane from becoming contaminated.
2. Load the gel slice into the Model 422 Electro-Eluter as described in Section 4.
3. Elute at 8-10 mA/glass tube constant current for 3 to 5 hours. Elution times may vary depending on gel percentage and the molecular weight of the protein.

4. After the elution is completed, remove the electro-eluter module from the buffer tank. Wear gloves to prevent contamination of the dialysis membrane.
5. Take the module, with the Glass Tubes attached, to a sink. If a stopper has been used, remove the stopper from the upper buffer chamber and allow the buffer to drain out. If there are no stoppers, carefully pull one glass tube out of the upper buffer chamber and allow the upper buffer to drain.
6. Using a plastic pipet, remove the buffer left in the tube to the level of the Frit and discard the liquid. Work quickly and carefully. Do not dislodge the Silicone Adaptor or the membrane cap. Make sure that the liquid below the Frit is not disturbed or shaken up during this process.
7. Remove the Silicone Adaptor together with the membrane cap from the bottom of the glass tube. The liquid level should be slightly above the membrane cap. Using a new plastic pipet, pipet the remaining liquid in the membrane cap into a microfuge tube. The volume should be approximately 400  $\mu\text{l}$ . With another 200  $\mu\text{l}$  of fresh elution buffer, rinse the membrane cap. Add the rinse solution to the microfuge tube. This solution will contain the eluted protein. Do this for each Glass Tube. The yield for eluted proteins is between 80-100%.
8. The membrane cap may be reused for at least five complete runs without decreasing the yield. Refrigerate the membrane cap in elution buffer with 0.05% sodium azide ( $\text{NaN}_3$ ). It is not necessary to reheat or resoak the membrane caps after the first use.

## 6.2 Elution of Fixed and Stained Proteins

With the Model 422 Electro-Eluter, it is possible to elute proteins that have been fixed and stained. If the fixing and staining process will not affect subsequent experiments, this is the preferred method. However, yields may be decreased slightly (10-20%) due to precipitation of proteins by methanol and/or cross-linking of the molecules by the dyes or stains. If bands are too closely spaced to allow estimation of their location in the gel by extrapolating from a stained reference lane (see Section 5.3), the following method should be followed to elute fixed and stained proteins.

1. Visualize the protein by staining the gel with Coomassie R-250 as described in Section 5.1.
2. Cut out the band of interest. Elute the proteins following the protocols in Section 6.
3. The yield of fixed and stained proteins is from 70% to 90%. Some protein may remain in the gel slice.

## 6.3 Concentration of Eluted Proteins Using Volatile Buffers

1. The Model 422 Electro-Eluter is assembled as described in Section 4. The proteins are visualized using one of the methods described in Section 5.1 or 5.2.
2. The protein is eluted from the gel as described in Section 6, except a volatile elution buffer is used. See Section 9 for recipes for the volatile buffer.
3. After the elution, the volatile buffer is lyophilized in a spin-vacuum, leaving concentrated protein. Elution yields using the volatile buffer are from 80% to 100%.

## 6.4 Elution of Proteins from Native Gels

Native proteins separated on non-denaturing slab gels can be eluted using the same native gel buffer system employed during electrophoresis in the slab (e.g. Ornstein-Davis buffer).<sup>1</sup>

Due to the absence of SDS in these buffer systems, expect the time required for elution to be approximately 3-6 hours.

## 6.5 Removal of SDS from Samples Subsequent to Elution

SDS can be removed from the buffer and/or the sample in several ways:

1. Dialysis: Both electro dialysis,<sup>2</sup> and equilibrium dialysis,<sup>2,3</sup> have been used for SDS removal. To electro dialyse, replace the lower buffer with fresh buffer made up without SDS approximately half way through the run.
2. Triton X-100: This may be added to the elution buffer at approximately 0.5% concentration. Triton can then be removed after the elution as per conventional methods.<sup>4</sup>
3. AG<sup>®</sup>11 A8 ion retardation resin: Using this ion-retardation resin, an average of 83% recovery of proteins may be expected, while 0.1 to 1.4 moles of SDS remain on each mole of protein.<sup>5</sup>
4. Anion exchange separation: AG1-X2 anion exchange resin<sup>6</sup> has been employed for SDS removal from proteins. Use of AG 1-X2 resin has resulted in 95% protein recovery with at most only 1% of all protein molecules still binding 1 molecule of SDS.
5. Bio-Beads<sup>®</sup> SM 2 beads. These macroporous support beads are mixed with the sample for SDS removal.

1. Ornstein, L. and Davis, B. J., *Ann N.Y., Acad. Sci.*, **121**, 321 (1964).
2. Tuszynski, G. P., and Warren, L., *Anal. Biochem.*, **67**, 55 (1975).
3. Visser, L., and Blout, E. R., *Biochem.*, **10**, 743 (1971).
4. Holloway, P. W., *Anal. Biochem.*, **53**, 304 (1973).
5. Kapp, O. H., and Vinogradov, S. N., *Anal. Biochem.*, **91**, 230 (1978).
6. Weber, K., and Kuter, D. J., *J. Biol.Chem.*, **246**, 4504 (1971).

## Section 7 Visualization of DNA

### 7.1 Visualizing DNA in Agarose Gels

1. Electrophorese the DNA in an agarose gel. Use high quality agarose to prevent agarose contaminants from affecting subsequent manipulations.
2. Stain the gel with an ethidium bromide solution. The concentration of the stain should be 0.5 µg/ml.

**Caution:** Ethidium bromide is a mutagen. Use gloves when handling ethidium bromide.

3. Place the stained gel on a UV light box. Cut out the band of interest with a scalpel.

### 7.2 Visualizing DNA in Acrylamide Gels

Often small pieces of DNA need to be eluted. For DNA of less than 1,000 base pairs, acrylamide gels are recommended.

1. Electrophorese the DNA in a polyacrylamide gel.
2. After electrophoresis, stain the DNA using ethidium bromide at 0.5 µg/ml in 1x TBE. Wear gloves when using ethidium bromide; it is a mutagen.

**Note:** Polyacrylamide quenches the fluorescence of ethidium bromide. Therefore, at least 10 ng of DNA/band must be loaded.

3. Excise the band of interest. Follow the Model 422 Electro-Eluter assembly procedures given in Section 4.

## Section 8 DNA Elution

### 8.1 Elution from Gel

1. Soak the membrane caps for at least 1 hour at 60 °C in DNA elution buffer. See Section 9 for recipes. Membrane caps may be soaked for longer than 1 hour. Wear gloves when handling the membrane caps to prevent the dialysis membrane from becoming contaminated. Use Green Membrane Caps MWCO 3500 daltons (catalog number 165-2986).
2. Assemble the Model 422 Electro-Eluter as described in Section 4.
3. The elution is done at ~10 mA/Glass Tube constant current. Elute the DNA for 15 minutes to 1 hour. The optimal elution time is dependent on the size of the DNA fragment being eluted. Elution times greater than the optimal time tend to drive the DNA into the dialysis membrane, making it difficult or impossible to retrieve the DNA.
4. After elution, reverse the polarity for approximately 1 minute to remove the DNA from the dialysis membrane. Take the electro-eluter module out of the buffer tank. Take the module, with the Glass Tubes attached, to a sink. If a stopper has been used, remove the stopper from the upper buffer chamber and allow the buffer to drain out. If there are no stoppers, carefully pull one Glass Tube out of the upper buffer chamber and allow the upper buffer to drain.
5. Using a plastic pipet, remove the buffer left in the tube down to the level of the frit and discard the liquid. Work quickly and carefully. Do not dislodge the Silicone Adaptor or

the membrane cap. Make sure that the liquid below the frit is not disturbed or shaken up during this process.

6. Remove the Silicone Adaptor together with the membrane cap from the bottom of the Glass Tube. The buffer level should be at the top of the membrane cap. Using a clean plastic pipet, pipet the liquid remaining in the membrane cap into a microfuge tube. The volume should be approximately 400  $\mu$ l. Rinse the membrane cap with 200  $\mu$ l of fresh elution buffer. Add this liquid to the centrifuge tube which contains the eluted DNA. The volume collected will be approximately 600  $\mu$ l. Do this for each Glass Tube.
7. Typical elution yields for DNA are between 70% and 90%. Some DNA will be irreversibly bound to the dialysis membrane, even if the polarity is reversed at the end of electro-elution.
8. Membrane caps should not be reused for DNA unless the same fragment is being eluted. Contamination of different DNAs will occur if a membrane cap is used for more than one fragment, because the DNA from the first elution will stick to the membrane.
9. If the same fragment of DNA will be eluted later, the membrane caps can be reused. Store the caps in DNA elution buffer with 0.05% sodium azide ( $\text{NaN}_3$ ) in the refrigerator. It is not necessary to reheat or resoak them after the first use.

## 8.2 DNA Purification

After the DNA has been eluted and collected, it will have to be purified for further use.

1. Add 1/10 volume 3M sodium acetate ( $\text{NaOAc}$ ) to the DNA sample. Add DNase free tRNA to the eluted DNA. The concentration of nucleic acids should be approximately 10  $\mu$ g/ml. The tRNA is added as a carrier to insure that all of the DNA will be ethanol precipitated.
2. Extract the DNA with an equal volume of equilibrated phenol. Vortex for at least 30 seconds. Spin in a microfuge for 2 minutes. Pull off the aqueous phase and put it in a new microfuge tube.
3. Extract a second time with an equal volume of phenol/chloroform. The chloroform is a 24:1 mixture of chloroform and isoamyl alcohol. Vortex for at least 30 seconds. Spin in a microfuge for 2 minutes. Pull off the aqueous phase and put it in a new microfuge tube.
4. Add 2 to 2.5 volumes of ice-cold ethanol. Place at  $-70^\circ\text{C}$  for one half hour, or overnight at  $20^\circ\text{C}$ .
5. Spin down for 15 minutes in a cold microfuge. Decant the supernatant.
6. Resuspend in 100  $\mu$ l of 10 mM Tris pH 7.6, 1 mM EDTA (TE). Repeat the ethanol precipitation. Decant the supernatant and rinse the pellet with 70% ethanol. Allow the sample to dry. Resuspend in TE.

## Section 9 Recipes

### 1. Protein Elution Buffer

Tris base	3.0 g	25 mM
Glycine	14.4 g	192 mM
SDS	<u>1.0 g</u>	0.1%
	to 1 liter with dH <sub>2</sub> O	

Store at 4 °C. Warm to 37 °C before use if precipitation occurs.

### 2. Volatile Buffer (for protein elution and concentration)

Ammonium bicarbonate (NH <sub>4</sub> HCO <sub>3</sub> )	3.95 g	50 mM
SDS	<u>1.0 g</u>	0.1%
	to 1 liter with dH <sub>2</sub> O	

Make up only 1 liter at a time because the buffer will volatilize. Store at 4° C.

3. DNA Elution Buffer	(50x)	Working Concentration
Tris	242 g	40 mM
Glacial acetic acid	57.1 ml	20 mM
0.5 M EDTA (pH 8.0)	100 ml	1 mM
SDS	<u>5 g</u>	0.1%
	to 1 liter with dH <sub>2</sub> O	

## Section 10 Troubleshooting Guide

SDS is included in elution buffer recipes to insure that the net negative charge of eluted molecules is maintained. As a result, the eluted molecules migrate toward the anode (positive electrode) and end up in the elution cup, SDS concentrates in the lower buffer chamber. If high SDS concentration is undesirable, at the end of the run the lower buffer can be exchanged for fresh elution buffer made without SDS, and the run continued for approximately 1/2 hour. This will effectively electro-dialyze SDS from the collected sample.

Proteins originally separated in native gels can be eluted without inclusion of SDS in the elution buffer. Because elution without SDS requires an approximately 25% increase in run times, and the volume of buffer contained in the Model 422 Electro-Eluter is small relative to a standard vertical slab electrophoresis apparatus, the buffer may require replenishment during these extended runs (very high MW proteins especially) to avoid "buffer depletion."

Buffer depletion is also problematic when running the eluter with more than the recommended 1 cm of gel per elution tube, which may require runs exceeding 6-7 hours. Even in the presence of SDS, extended runs (6 hours to overnight) will require at least one change of upper and lower elution buffer. The buffering capacity of the elution buffer maintains the pH at which molecules maintain electrophoretic mobility. Alkaline pH (8-9) is standard for most Tris-glycine or phosphate based native electrophoresis/elution buffers.



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