

Hoefer IsoPrime

IEF Purification Unit

User Manual

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1

IsoPrime Function

The PI 8 IsoPrime Multi-chambered Electrofocusing unit uses 'pI selective' membranes together with electrophoresis to purify proteins on a preparative scale. The amount of material purified and the time required depend on the solubility of the proteins and the degree of resolution sought.

The IsoPrime comprises a separation module, a sample circulation system and a safety enclosure. The separation module has electrodes at each end, and is divided into a series of up to eight separation chambers by glass fiber filter-reinforced polyacrylamide membranes. The membranes covalently incorporate acrylamido buffers to fix a series of isoelectric pI points that define steps in a pH gradient. The peristaltic pump circulates the material to be separated from sample vessels through the separation chambers, where electrofocusing takes place. Proteins move by electrophoresis through the isoelectric membranes until they reach a chamber bounded by membranes with pIs which bracket the pI of the protein. With appropriately selected membrane pIs, each chamber and the sample vessel it is connected to will contain an isoelectrically homogeneous fraction at the end of the run.

Important safety information

- ◆ This instrument and its components are for *in vitro* research use only.
- ◆ Warning: Operation of this instrument requires potentially hazardous high voltage. It should be used only by trained persons. Turn off and disconnect any high voltage power supply before opening the safety enclosure lid. The high voltage safety interlock is not to be used as the primary high voltage switch for the instrument.
- ◆ Warning: If liquid is observed to have spilled or overflowed during a separation run, turn off the high voltage power supply and the AC mains power to the entire instrument before opening the safety enclosure lid.
- ◆ Disconnect any instrument from the AC mains before cleaning or servicing.
- ◆ Acrylamide is a neurotoxin. Wear gloves and work under a fume hood when handling.



Caution! Refer to the manual.

2

Assembly and Installation

Unwrap all packages carefully and compare contents with the packing list, making sure all items arrived. If any part is missing, contact Hoefer. Inspect all components for damage that may have occurred while the unit was in transit. If any part appears damaged, contact the carrier immediately.

Be sure to keep all packing material for damage claims or to use should it become necessary to return the unit.

Following is a brief description of the main IsoPrime components and a guide to where to find detailed assembly instructions.

2.1 Cabinet components

Figure 1 shows the IsoPrime main components. On the right side of the cabinet is a platform that supports both the separation module, which plugs into the electrode posts in the rear, and the sample vessel tray, which rests on the stirring platform in the front.

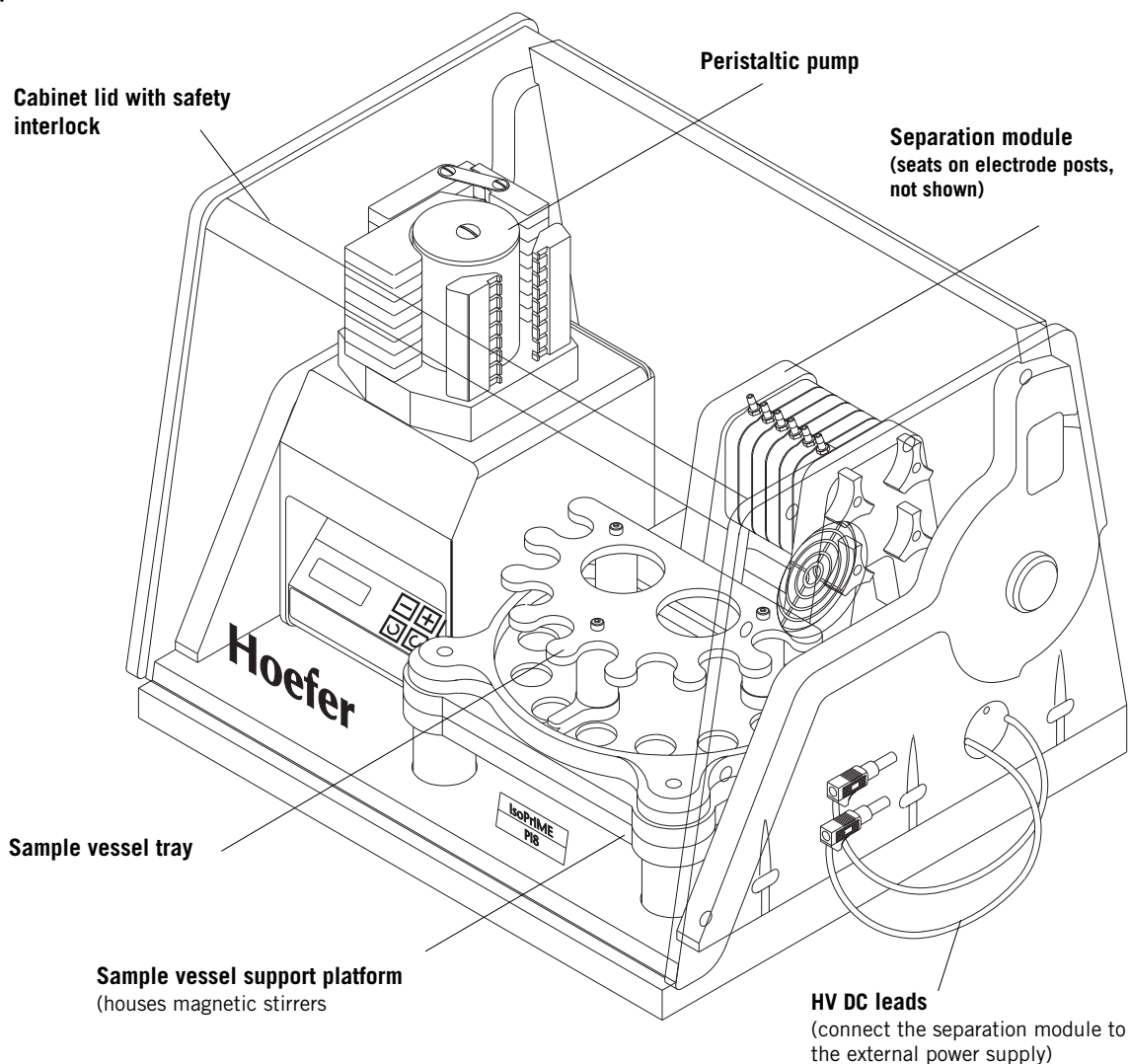
Separation module. Fit the pre-assembled separation module onto the electrode posts. Orient the module so that the end with the black knobs, labeled "basic -", fits onto the black cathode post and the end marked "acidic +" fits onto the red anode post. To prepare the module for a purification run, see Section 5.1, "Assemble the separation module."

Sample vessel tray. The sample vessel tray fits onto the stirring platform by seating onto two screw heads that protrude near the front of the platform. No further installation is required. The sample vessel tray holds up to eight 30-ml vessels and two 125-ml vessels.

Sample vessels and tubing. (Not shown). A peristaltic pump circulates the sample between the sample vessels and separation chambers. To assemble the fluid paths, see Section 5.1, "Create a fluid path for each separation chamber."

Peristaltic pump. The pump fits into the left side of the cabinet, where its rubber feet fit into matching depressions in the cabinet bottom. With the pump in place, tilt it forward slightly to plug the short power cord in the back of the IsoPrime cabinet into the pump's power cord receptacle. See section 2.4 for complete pump operating instructions.

Figure 1.
IsoPrime main components



Auxiliary parts supplied but not shown

- Peristaltic pump tubing
- Sample vessel tubing
- Numbered tubing clips (3)
- Mains power cord
- Sample vessels, 30 ml, (12) and caps (8)
- Dual membrane casting chambers (8) and Glass covers (16)
- Whatman GF/D 4.7 cm dia filters (box/100)
- Doctor pH software and User's Manual

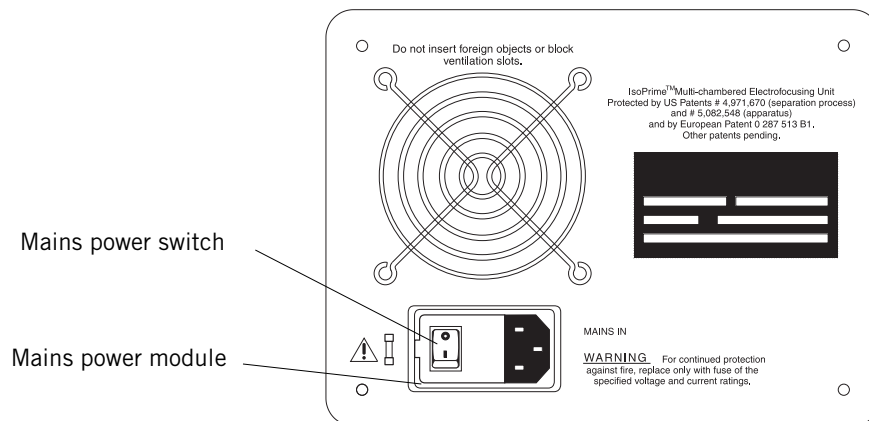
Materials required but not supplied

- Power supply (3000 V recommended)
- Acrylamido buffers (10 buffers are commercially available: pK's 1.0, 3.1, 3.6, 4.6, 6.2, 7.0, 8.5, 9.3, 10.3, >12)
- Acrylamide and bisacrylamide
- TEMED
- Ammonium persulfate
- Light paraffin oil
- Repel-Silane

2.2 Power

Mains power module. The IsoPrime mains power module, located in the back of the unit, houses the mains power switch and two fuses. The mains power switch is the only operating control on the instrument. It supplies power to the peristaltic pump, the fan, the stirring platform, and the HV safety interlock. Refer to Section 7.3 Care and Maintenance for instructions for replacing fuses.

Figure 2.
IsoPrime back panel



Separation module power supply. An external power supply is required to operate the separation module. The maximum rating of the power supply used with the IsoPrime must not exceed 4000 V, 50 mA, and 15 W.

The 2-mm safety leads at the right side of the instrument connect the separation module to the power supply. If your power supply does not accept 2-mm safety leads, adapters (2-mm lead to 4-mm output) are included with the unit. **If your 4-mm power supply output is recessed**, plug the short adapter (code no. 80-6105-84) into the 2-mm safety lead and then plug this assembly into the output. **If your 4-mm output is not recessed**, plug the longer adapter ((code no. 80-6105-46) onto the short adapter (code no. 80-6105-84), plug both into the 2-mm safety lead, and then plug this assembly into the power supply output.

Note

Illustrated instructions accompany the safety lead adapters.

2.3 Doctor pH software

Doctor pH software calculates buffer compositions for any specified membrane pH and for pH gradient gel mixtures. The program requires Windows 3.0 or later and a minimum of an 80286 microprocessor, but an 80486 or an 80386 with a math coprocessor is strongly recommended. For easy reference, the program includes an on-line Help file and a separate user's manual.

2.4 Peristaltic pump

The peristaltic pump uses a hybrid stepper-motor to deliver fluids with high speed stability and a low pulsation level. The pump head is composed of a rotor with stainless steel rollers that press against flexible tubing held in place by compression cams. The pump is controlled by a front-panel keypad.

Figure 3.
Peristaltic pump

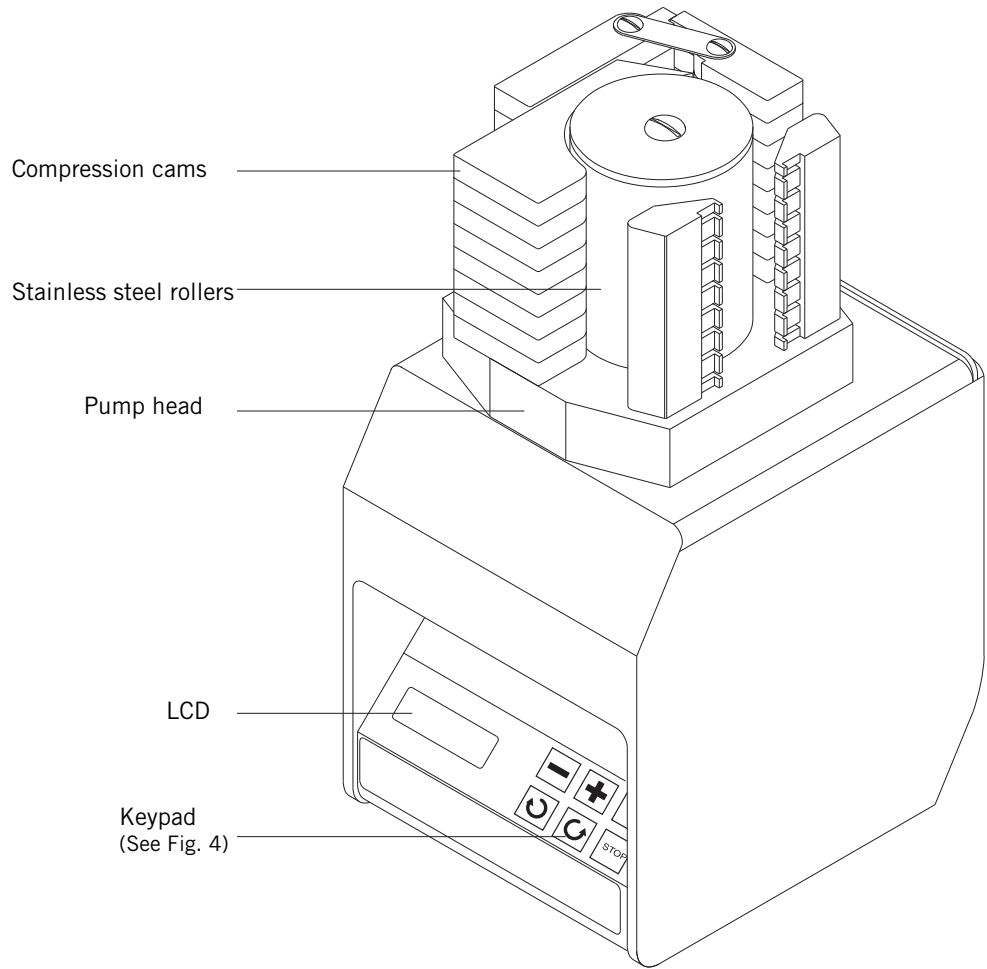
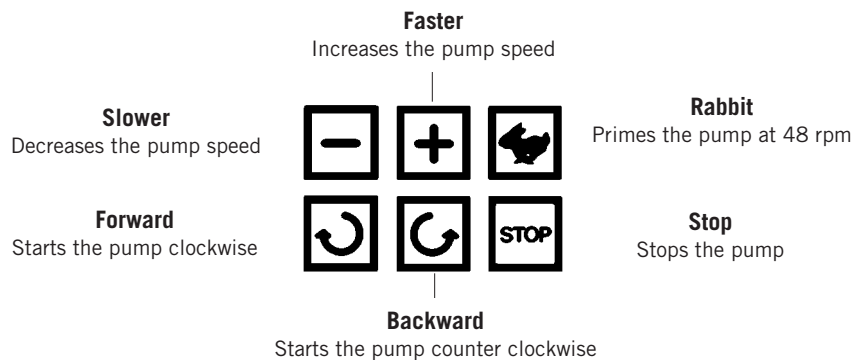


Figure 4.
Pump keypad functions

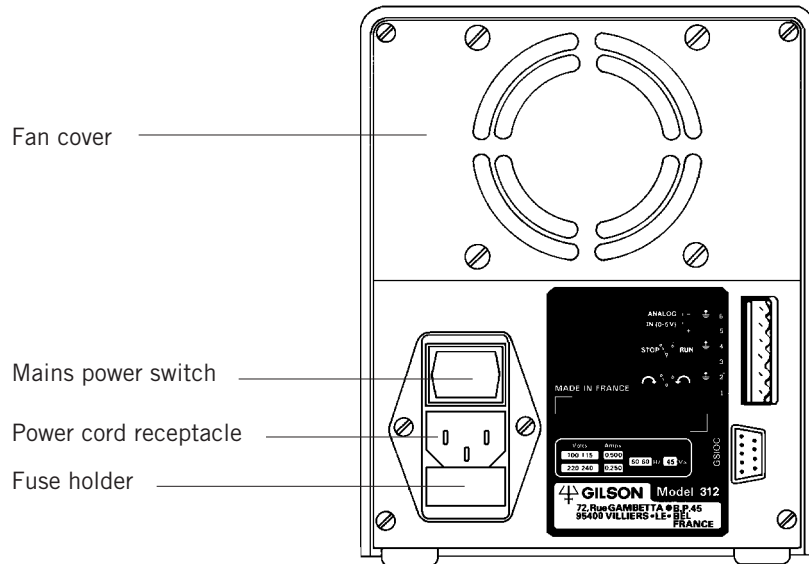
The 6 keys on the keypad control the pump. The 3-digit Liquid Crystal Display (LCD) to the left of the keypad displays the speed (in rpm) at which the pump is running.



Pump mains power

The back panel houses the mains power module, which contains the mains power switch, a power cord receptacle, and the fuse holder. Turn the pump on by turning the mains power switch on. (The IsoPrime mains power switch must also be on in order for the pump to operate.) Turn the pump mains power off when the pump is not in use.

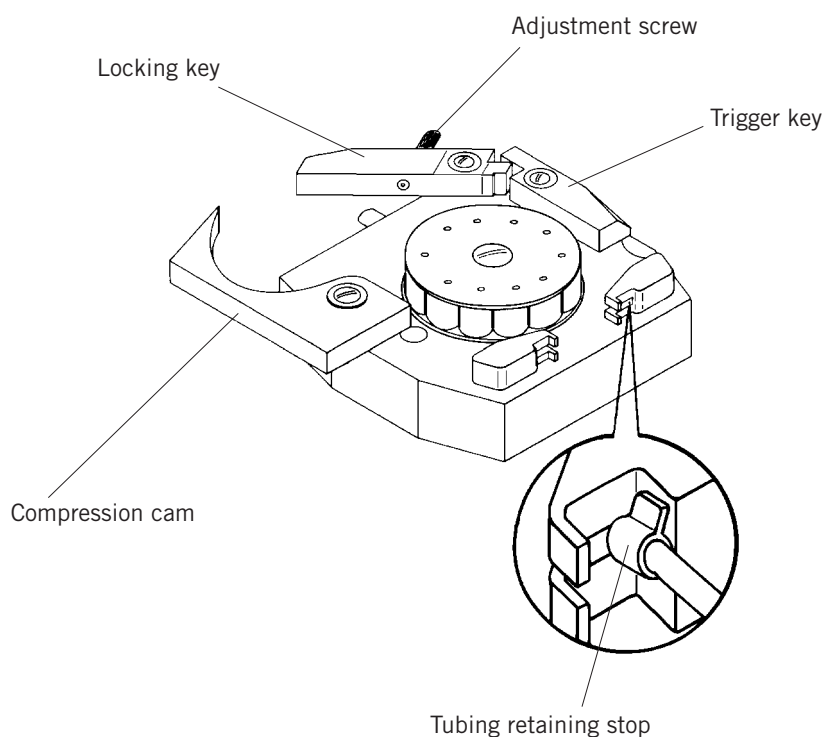
Figure 5.
Pump back panel



Peristaltic tubing installation

The pump head accommodates from 1 to 8 lengths of peristaltic tubing. (Install one tubing length per separation module chamber.) Each tubing length has 2 retaining stops (one black, one purple) that correctly tension the tubing on the pump head. Refer to Figure 11 in section 5.1 for the complete fluid flow path, which includes the tubing position around the pump head. To install tubing:

Figure 6.
Pump head components



- 1 Unlock all 8 pump head channels by pressing the trigger key of each section toward the roller barrel.
- 2 Starting at the bottom of the pump head, install one length of tubing at a time by sliding the black retaining stop into the front stop, wrapping the tubing around the roller barrel, and sliding the purple retaining stop into the back stop.
- 3 Swing each compression cam toward the roller barrel and close the locking key. Check that the top and bottom lip of each channel centers the tubing.

Important

To increase tubing life, release pressure on the tubing when the pump is not in use: Release the compression cams by pressing on the beveled corners of the trigger key, and slacken the tubing by unhooking one of the retaining stops.

Pump operating instructions

Preliminary pump set up includes placing the pump into the left side of the cabinet, plugging the pump power cord into the pump, installing the both the peristaltic and the sample vessel tubing, and switching the IsoPrime mains power switch on.

Controlling the keypad functions (The keypad is shown in Figure 3)

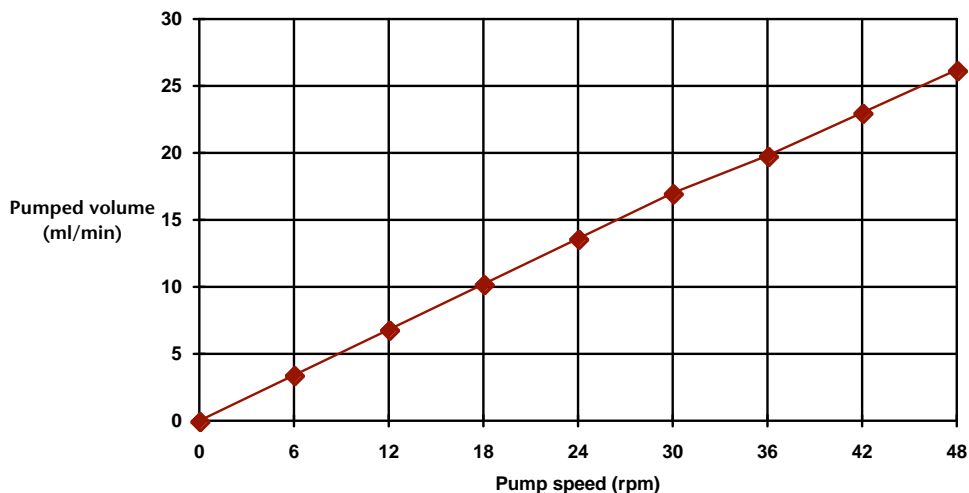
- 1 Switch the pump mains power on. The LCD shows the pump speed, which is the speed set before the pump was last switched off. (The speed is factory set at 12.5 rpm.)
- 2 **To change the pump speed**, press the (-) key to decrease the displayed value, or the (+) key to increase the displayed value. Pressing either key once changes the right-hand digit by one unit (rpm). Continuously pressing the key accelerates the change in speed. To deliver 3 to 4 ml per minute, set the pump speed to 6.5 rpm. Figure 7 below shows the flow rate (ml/min) at pump speeds up to 48 rpm. Figure 7 below shows the flow rate (ml/min) at pump speeds up to 48 rpm.
- 3 **To start the pump**, press the arrow key showing the desired direction of flow. A (+) sign will then appear at the left of the speed value for clockwise rotation or a (-) sign for counter clockwise rotation. Once the pump is started, pumping direction can be changed without stopping the pump.
- 4 **To purge or to prime the tubing**, select the RABBIT key when the pump is running. Pressing the RABBIT key runs the pump at its maximum speed of 48 rpm in the current direction. When pressed, the display shows (+ - - -) or (- - - -), but the previous speed is stored in memory. If the RABBIT key is pressed again or if a directional key is selected, the pump runs at the previously set speed.
- 5 **To stop the pump**, press the STOP key. The last selected speed (except the RABBIT key selection) is stored for the next start.

Note

If power is suddenly turned off while the pump is operating, the display will flash until any key is pressed. The pump will then resume operating at the same speed as before.

Figure 7.

Flow rates at pump speeds up to 48 rpm.



Adjusting fluid flow rate

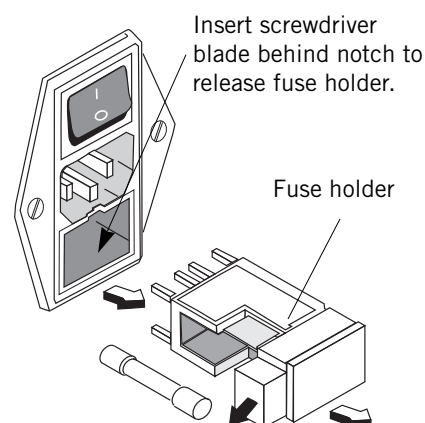
Once the pump is running, adjust the cam pressure on the tubing to the minimum necessary to ensure liquid flow. Adjust compression cam pressure by slowly tightening the adjustment screw with your thumb and index finger until you can see liquid being pumped inside the peristaltic tubing. Then tighten the screw another 1/8 turn. Do not over-tighten the screws. Readjust after the pump has operated 15 minutes. (Figure 6 shows adjustment screw location.)

To ensure that consistent and accurate flow rates are maintained, replace peristaltic tubing as soon as it appears to be worn.

Replacing pump fuses

Two fuses are factory installed into the fuse holder in the pump mains power module. Fuses do not require maintenance, but may fail under normal operating conditions. To replace fuses:

- 1 Turn the mains power switch off, disconnect the power cord, and lift the pump out of the IsoPrime cabinet.
- 2 Insert a screwdriver blade into the notch above the fuse holder and twist it a 1/4 turn to partially pull out the holder. Then pull the fuse holder completely out of the mains power module.
- 3 Pry one fuse out of one side of the fuse holder by inserting the screwdriver blade into the opening on the bottom of the holder. Inspect the fuse element. If it is burned or broken, replace the fuse with an identical type. If the fuse appears to be intact, check it with an ohmmeter. (A short circuit indicates the fuse is still usable.)
- 4 Slide the drawer in the fuse holder out, insert a new fuse, and slide the fuse into the fuse holder, making sure it fits securely into both clips at either end.
- 5 Repeat steps 3 and 4 for the second fuse on the other side.
- 6 Reinsert the fuse holder so that the line voltage supplied to the instrument reads right side up. (The arrow next to voltage rating points to the alignment mark at the bottom of the pump mains power module.)



To install a new fuse, slide drawer out and insert fuse. Then slide drawer in. Snap fuse into clips.

Figure 8. Pump mains power module

Replace the fuse holder so that the line voltage supplied to the instrument (110 V or 230 V) reads right side up.

3

Purification Method Summary

The PrIME process has been applied to purifying a variety of proteins, and certain procedures are recommended based on this experience. Listed below are important reminders, method capabilities, and also some limitations.

Important

- ♦ The intent of this process is to purify proteins. It is therefore imperative to follow all instructions carefully, prepare all solutions with the utmost attention to accuracy, and take all precautions to avoid contamination during the entire process.
- ♦ Analytical and separation conditions should be as consistent as possible. If additives are required to maintain isoelectric solubility, use the same additives at the same concentrations during analytical pI determination and preparative separations. Use the same set of acrylamido buffers for analytical gels and membranes. If you cast your own analytical IPG gels for pI determination based on Doctor pH calculations, use the "Instantaneous Value" feature to create membranes from the same set of buffers for the preparative separation.

Capabilities

- ♦ Resolves isoforms of a protein of a single molecular mass.
- ♦ Resolves degradation and modification products of proteins.
- ♦ Resolves proteins with a molecular mass <300 kD.

Limitations

- ♦ Salts cannot be used to solubilize proteins.
- ♦ Enzymes must be stable in a pH environment close to their pI points.
- ♦ Proteins with a molecular mass >300 kD are too large to move through the membranes. A system of membranes can usually be designed that causes smaller species to migrate away from the larger species.

Purification strategy overview

Detailed descriptions of IsoPrime procedures follow in Section 4.

- 1 **Use conventional methods to purify the protein of interest to 50-70% pure by SDS PAGE analysis.**

PrIME is best used in the later stages of a purification procedure. In some cases crude samples can be processed, but minimally purified samples can exhibit serious problems with isoelectric precipitation and non-specific aggregation.

- 2 **Desalt the sample as completely as possible.**

Proteins do not migrate until all salt ions have moved into the anode and cathode chambers. A high salt content (>50 mM) can also cause membranes to rupture due to the high electric current they carry.

- 3 **Dissolve or dialyze the protein into distilled water if possible.**

If the protein is not soluble in distilled water, add solubilizing agents such as non-ionic detergents, glycerol or urea. The best solubilization conditions and combinations should be determined by UV absorbance, HPLC or IEF measurements of protein solubility.

- 4 **Determine the pI of the protein and select membrane pHs.**

For the most accurate results, cast and run one or more linear immobilized pH gradient (IPG) gels. Use linear interpolation to estimate the pI of the proteins and contaminants, and to estimate the best pHs for separating membranes. Properly selected membrane pHs will separate the desired protein and contaminants into different chambers.

- 5 **Prepare isoelectric membranes.**

Calculate acrylamido buffer mixtures for each pH membrane needed with Doctor pH software.

- 6 **Pre-run and load the IsoPrime.**

Assemble the separation unit with the membranes, fill the system with the separation solution, then pre-run. Load the protein sample of interest and begin the purification run.

4**Preliminary Purification Procedure****4.1 Partially purify the protein of interest**

The protein sample should be more than 50% pure (as determined by SDS-PAGE) after using conventional purification techniques such as precipitation/centrifugation, filtration, and column chromatography. Crude, desalted protein samples can be purified with the IsoPrime, but results are unpredictable because crude mixtures tend to precipitate due to nonspecific aggregation.

4.2 Desalt the sample

The sample must be desalted before loading into the IsoPrime. High conductivity samples lead to high currents and excessive joule heating in the separation module, membrane rupture, and retarded protein separation. As salts of strong acids or bases move in the electric field, they form strongly acidic and basic fronts that can cause protein denaturation due to pH shock.

Use conventional desalting techniques, such as dialysis, ultrafiltration, gel filtration, ion-exchange chromatography, ultracentrifugation, desalting columns, or buffer replacement in a centrifugal concentrator. If it is not possible to completely desalt the protein sample, use buffers formed from weak acids and bases, such as Tris-acetate. Avoid salts of strong acids and bases such as NaCl or phosphate.

Do not use protein samples previously exposed to ionic detergents such as SDS. As with simple salts, ionic detergents cause high initial electric currents and may cause membrane rupture.

4.3 Stabilize the protein in solution

Some proteins are adequately soluble in distilled water, the preferred solvent for PrIME purification. However, for proteins that have low solubilities, or tend to aggregate or precipitate, a number of useful "solubilizing agents" are listed below. Solubilizing agents are also helpful in reducing losses due to non-specific adsorption when working with proteins available in very limited quantities. Determine the best combination and concentration of additives using techniques such as UV absorbance, HPLC, HPCE or IEF to examine protein solubility under the various conditions. Once effective solubilizing conditions are determined, use them for all PrIME-related procedures, including pI determination and preparative runs.

Important: Do not use ionic detergents such as SDS to solubilize proteins.

Refer to the IsoPrime application notes for solubilization procedure examples.

Table 1.
Non-ionic solubilizing agents that can be used with the IsoPrime.

Non-ionic detergents	Concentration
CHAPS	1-2%
CHAPSO	1-2%
Digitonin	1-2%
NP-40	1-2%
Tween 20	1-2%
Zwittergent	1-2%
β -Octyl glucoside	1-2%
n-Octyl- β -D-glycopyranoside	1-2%
Other solubilizers	
Urea* (for acidic range only)	up to 8 M (up to 6 M at 4 °C)
Tetramethyl urea	up to 6 M
Sorbitol/Mannitol/Trehalose	up to 40%
Glycerol	5-40%
Ethylene glycol	5-40%
Propylene glycol	5-40%
Dimethyl sulfoxide (DMSO)	up to 40%

*Urea should not be used as a solubilizer at alkaline pHs for long periods, as this can result in carbamylation of the protein.

Note 1 Evaluate the protein sample solubility in free solution. Its solubility may be different in other media, such as an analytical gel or a carrier ampholyte IEF gel. The solubility of certain concentrated protein samples has been observed to be more stable in a gel matrix than in a solution.

Note 2 Proteins may degrade or change conformation with time (for example, due to disulfide scrambling). Add 2-mercaptoethanol at acidic pHs to help resububilize oxidized proteins.

4.4 Determine the pI of the protein

The most accurate technique for determining the pI of proteins is to run one or more immobilized pH gradient (IPG) gels and interpolate the pI of the protein from its position in the gradient. Two less accurate alternatives to this method are: run other types of gels, or systematically narrow down the pI of the protein sample using iterative IsoPrime runs. Each method is described below.

IPG gels

IPG gels, especially if you formulate them with Doctor pH and then use the same set of acrylamido buffers and solubilizing agents in all solutions throughout the process, are the most accurate option for determining the pI of proteins. In addition to higher accuracy, IPG gels also handle protein loads of up to 10-20 times more than a conventional carrier ampholyte (IEF) gel. This level of resolution helps identify contaminants more easily.

Pre-cast Immobiline analytical gels are available in the acidic pH range. Gradient mixtures for casting your own gels are easily calculated with Doctor pH software. Once the gel is prepared:

- 1 Load the protein sample onto one or more IPG gels. (Include pI markers to help detect irregularities in the gel, particularly when solubilizing agents are used.) Run the gel(s) at the same temperature that the purification will be run—2.5 to 3.0 °C above cold room or laboratory temperature—because temperature can affect the apparent pI of a protein.

Note: If standard bands are sharp, but experimental bands appear smeared, proteins may be interacting or precipitating. Find an appropriate solubilizing agent and use it in the same concentration throughout the procedure because it can affect the apparent pI. Refer to Section 4.3, the application notes, or the Immobiline gel instructions for more solubility information.

- 2 Determine the pI values of the protein by measuring the distance between the protein of interest and the pH gradient boundaries. Interpolate the pI value.

Note: Because the pI values of the markers were determined under different conditions than the conditions of your gel run, markers may indicate a pI value different from the interpolated results. If there is a discrepancy, use the values from your interpolation.

- 3 Refine the pI estimate by casting increasingly narrower pH span gels until the desired degree of resolution is achieved.

Important

If CA's are used, the concentration should not exceed 0.3%, and the analytical gels should contain the same CA concentration as the sample to be purified.

Carrier ampholyte (CA) flatbed IEF gels, protein sequencing

It is possible to determine the pI of the proteins in a sample using commercially available CA flatbed IEF gels, but the resulting accuracy and resolution is lower than the results achieved with analytical IPG gels, especially in the alkaline pH range. The presence of carrier ampholytes may result in an apparent pI value differing as much as 1.0 pH unit from the actual value (due to potential CA/protein complexing). This effect is amplified in the alkaline range.

It is possible to calculate a pI from a known protein sequence, but the calculated value is rarely useful unless the PrIME separation is performed under denaturing conditions. Theoretical pI calculations cannot account for conformational effects on pI.

Systematic pI determination with IsoPrime (iterative method)

To determine the pI of a protein using the IsoPrime, run the sample through a series of membranes, beginning with a broad range and systematically narrowing the range. (Refer to Sections 4.5, 4.6, and 5 (all) for membrane casting and IsoPrime operation instructions.)

For example, the membrane pH values in the first run may be 4.0, 4.5, 5.0, 5.5, 6.0, and 6.5. If all the proteins of interest migrate to the chamber delimited by the membranes with pH values of 5.0 and 5.5, then the pHs of the next set of membranes would be 5.0, 5.1, 5.2, 5.3, 5.4, and 5.5. Continue to narrow the range by casting membranes with increasingly closer spaced pH values until the desired degree of purity is achieved. (Analyze the purity of the separated sample by SDS-PAGE, IEF, or immunological techniques.)

4.5 Prepare isoelectric membranes

Determine membrane pH values

The pH of each membrane set is selected so that the different molecules in the solution (whose different pI values are known) are trapped in separate chambers. For most cases, ideal pH values are those that are spaced to allow the maximum difference between the pI values of the molecules in the solution and the pH values of the membranes. This is illustrated in the following example:

A sample contains five proteins with pIs of 5.20, 5.30, 5.40, 5.50, and 5.60. Four membranes with pHs of 5.25, 5.35, 5.45, and 5.55 are optimal because these values allow the largest difference between the pI values and the pH values. Other sets of membranes within this range would also serve to purify this sample. Only 4 membranes are specified because the pI=5.20 protein will migrate to the anode chamber, and the pI=5.60 protein will migrate to the cathode chamber. See the IsoPrime application notes for other examples.

Select membrane buffer set

A membrane of practically any pH can be made from just two acrylamido buffers: one with a pK value close to the pH of the membrane, and another with a much higher (or lower) pK value to titrate the solution to the desired pH.

If possible, it is advisable to use one buffer with a pK value slightly higher and another buffer with a pK value slightly lower than the pH of the membrane in order to bolster the buffering capability on both sides of the membrane pH. (As opposed to using only one buffer and a strong titrant, which would result in limited buffering capability on the titrant side of the pH.) The buffering capability and ionic strength of a membrane can be specified by using the appropriate range of buffers.

The buffering capacity of any membrane with acrylamido buffers is several thousand times higher than the protein solution in terms of buffering and titrant molarity. These properties cause the membranes to isolate the protein in the appropriate chamber and keep the protein isoelectric.

Listed below are all commercially available acrylamido buffers and each buffer's type, pK value, and molecular mass, (Mr, relative to H).

Table 2.
Acrylamido buffer characteristics

Acrylamido buffer	type	pK	Mr
2-Acrylamido-2-methylpropane sulfonic acid	acid titrant	1.0	207
2-Acrylamidoglycolic acid	acid	3.1	145
N-Acryloylglycine	acid	3.6	129
4-Acrylamidobutyric acid	acid	4.6	157
2-Morpholinoethylacrylamide	base	6.2	184
3-Morpholinopropylacrylamide	base	7.0	198
N,N-Dimethylaminoethylacrylamide	base	8.5	142
N,N-Dimethylaminopropylacrylamide	base	9.3	156
N,N-Diethylaminopropylacrylamide	base	10.3	184
N,N,N-Triethylaminoethylacrylamide	base titrant	>12	198

Available as Immobilines from Amersham Pharmacia Biotech or as acrylamido buffers from Fluka Biochemika

Calculate acrylamido buffer concentration

The easiest (and most accurate) way to calculate the concentration of acrylamido buffers required for any specified membrane is to enter the acrylamido buffer types and pK values into a Doctor pH software membrane worksheet, specify the buffering power, and allow the program to compute the concentrations. (Refer to the Doctor pH manual for more detailed instructions.) Alternatively, request tables and nomographs from Amersham Pharmacia Biotech to manually calculate concentrations.

Prepare membrane stock solution

Note

Use utmost care to follow all procedures precisely and to avoid all contamination. Proper membrane preparation is essential to ensure effective and reproducible protein separations.

Preparation of isoelectric membranes is simpler and more reproducible with a set of stock solutions. Acrylamido buffers are provided by the manufacturer as 0.20 M solutions in water or isopropanol. Commonly used acrylamide/bisacrylamide stock solutions are listed in Table 3. (The composition of any polyacrylamide gel is given by two parameters: %T, which represents the total concentration in grams per 100 ml of monomer used to produce the gel (acrylamide plus bisacrylamide), and %C, which is the percentage (by weight) of the total monomer that is the crosslinking agent.)

The choice of final acrylamide/bis concentrations and ratios will vary depending on the size of the proteins and impurities to be separated, and the position of the membrane relative to the electrode. For most applications, 5%T/4%C is suitable for internal membranes and 10%T for membranes adjacent to the electrodes. Dilute all stock solutions to final concentration with double distilled water.

Table 3.

Recipes for 30% T/x% C Acrylamide /bisacrylamide stock solution

Final conc.	acrylamide (g)	bisacrylamide (g)	dd H ₂ O
30%T/4% C	57.6	2.4	to 200 ml
30%T/5% C	57.0	3.0	to 200 ml
30%T/6% C	56.4	3.6	to 200 ml
30%T/8% C	55.2	4.8	to 200 ml
30%T/10% C	54.0	6.0	to 200 ml

Cast the membranes

Important

Always wear gloves when handling membranes, both for your protection and to avoid contaminating the membranes.

Handle the membranes as little and as gently as possible to avoid damaging them.

Important

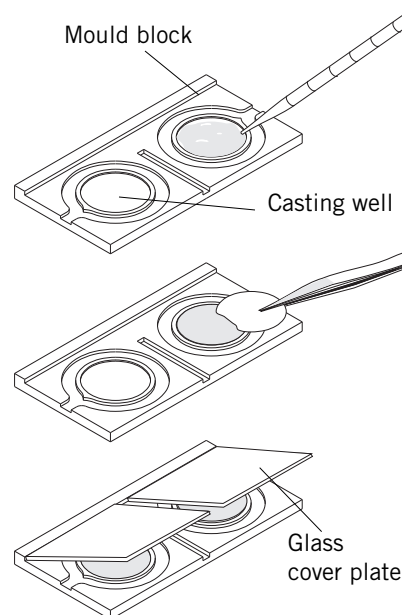
Prepare and cast only one membrane solution at a time in order to prevent premature polymerization.

Figure 9.
Membrane casting chambers

- 1 Prepare, deionize, filter, and degas enough acrylamide/bis monomer stock solution for a minimum of 10 ml final diluted volume for each membrane pH value. (Recipes are listed in Table 3.)
- 2 Remove acrylamido buffers from 4 °C storage and allow to warm to room temperature for 30 minutes.
- 3 Cover the work area with absorbent paper. Label one membrane mould block (2 casting wells, each 1 mm x 4.7 cm dia. with glass cover plates) for each pH membrane formulation. (Always make two membranes and use the best one.)
- 4 Coat the clean, dry, cover plates for each mold block with »1 ml of Repel-Silane solution and allow to dry several minutes. Repel-Silane (2% dichlorodimethyl silane in 1,1,1 chloro-trichloroethane) prevents membrane adhesion to the cover plates.
- 5 Copy Worksheet 1 (next page). For each membrane pH, list the acrylamido buffer concentrations calculated by Doctor pH in the conc. column. Then calculate the exact volume of each buffer needed for 10 ml of membrane solution by multiplying the buffer concentration by 50 to yield the volume in ml.
- 6 Follow the protocol listed on the lower portion of the worksheet. Be sure to measure the acrylamido buffer solution pH before adding the acrylamide/bis stock solution. If the pH is not 6.5 (± 0.2 pH units), adjust it to this range with 1M Tris base or 1M acetic acid to optimize polymerization.
- 7 Prepare 1 ml of 40% ammonium persulfate (APS) immediately before use. (0.4 g APS in 1 ml of ddH₂O.) Add acrylamide/bis stock solution, 5 μ l of TEMED, and 10 μ l of 40% APS to the acrylamido buffer solution. Mix thoroughly with a transfer pipette.
- 8 Deliver 3 ml of the solution into each of the 2 wells in the mould block.

Wet a Whatman GF/D, 4.7 cm filter by placing it at an angle into a well and allowing capillary action to saturate it with the solution. Once saturated, lower the filter into its well, gently pressing it into place with gloved fingers. The filter must settle into the casting well so that the filter edges are completely within and evenly centered in the well. Pipette an additional 2 ml of solution onto each filter.

- 9 Slowly lower the glass cover plate, with the silane-coated side toward the membranes, allowing excess gel solution to escape from between the plate and the acrylic block. Avoid trapping air under the cover plate. Air retards polymerization, and air bubbles may cause holes in the membrane.



Worksheet 1. Protocol for preparing 2 membranes at each pH used in the purification of _____

A Convert Doctor pH concentrations (mM) into volume (μ l) of acrylamido buffer: **Multiply conc. by 50.** (Multiplier based on using 200 mM acrylamido buffers to make 10 ml of membrane solution.)

Membrane	1		2		3		4		5		6		7	
Membrane pH (calculated)	conc mM	vol μ l	conc	vol	conc	vol	conc	vol	conc	vol	conc	vol	conc	vol
pK 1.0	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 3.1	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 3.6	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 4.6	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 6.2	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 7.0	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 8.5	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 9.3	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 10.3	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK >12	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
B Fill ddH₂O to 2.000 ml		2.000 ml		2.000		2.000		2.000		2.000		2.000		2.000
C Add acrylamido buffer vol.														
D Fill ddH ₂ O to 6.000 ml, mix		6.000 ml		6.000		6.000		6.000		6.000		6.000		6.000
E Measure solution pH		_____		_____		_____		_____		_____		_____		_____
F Adjust pH to 6.5, \pm 0.2 with 10-100 μ l 1M Tris base or 1M acetic acid, mix		6.5		6.5		6.5		6.5		6.5		6.5		6.5

Important: For the following steps, prepare only 1 membrane solution at a time and pour into mould before starting the next solution.

%T/%C	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
G Add acrylamide/bis stock (30%T) _____ μ l		_____		_____		_____		_____		_____		_____		_____
H Fill ddH ₂ O to 10.00 ml, mix		10.00 ml		10.00		10.00		10.00		10.00		10.00		10.00
I Add TEMED (μ l)		5 μ l		5		5		5		5		5		5
J Add APS, 40% (w/v) (μ l), mix		10 μ l		10		10		10		10		10		10

Pour into mould. Do not disturb for 20 minutes.
 Polymerize for 1 hr at 50°C or overnight at room temperature.
 Disassemble and start washing procedure.

Polymerization and storage

- 1 Do not disturb membranes for 20 minutes. After 20 minutes either carefully transfer the gel cassettes to a forced-ventilation oven set at 50 °C and leave for 1 hour or leave the cassettes undisturbed at room temperature overnight.
- 2 After polymerization, gently pry the gel cassette open with a thin piece of plastic or a spatula. Carefully trim excess gel from the membrane edges with a sharp blade or spatula.
- 3 Thoroughly examine each membrane for even polymerization: the surface of each membrane should be smooth and no holes should be evident. If the membranes are acceptable, wash them in a small dish three times. Use 100 ml ddH₂O per wash and leave each wash on the shaker for 30 minutes. (Label each wash container with the pH of the membrane.)
- 4 Store unused membranes for up to 1 month in 20 mM sodium acetate buffer, pH 5.0, 2 mM sodium azide. Wash the membranes with ddH₂O for 30 minutes before using.

4.6 Prepare the sample

The stabilized protein sample (section 4.3) may need to be diluted or concentrated before it is purified in the IsoPrime. For most proteins, a concentration between 1 and 10 mg/mL is an appropriate starting point. At less than 0.1 mg/ml, losses to non-specific surface adsorption may substantially decrease protein recovery, although losses may be reduced by addition of solubilizing agents. Some proteins may precipitate at concentrations >10 mg/ml. Although the starting concentration may not be high enough to cause precipitation, sample concentration and precipitation may occur during a run, particularly if the starting sample is introduced in several sample vessels but concentrates in one. See "Troubleshooting" for suggestions on dealing with precipitation problems.

5

Purification Run Instructions

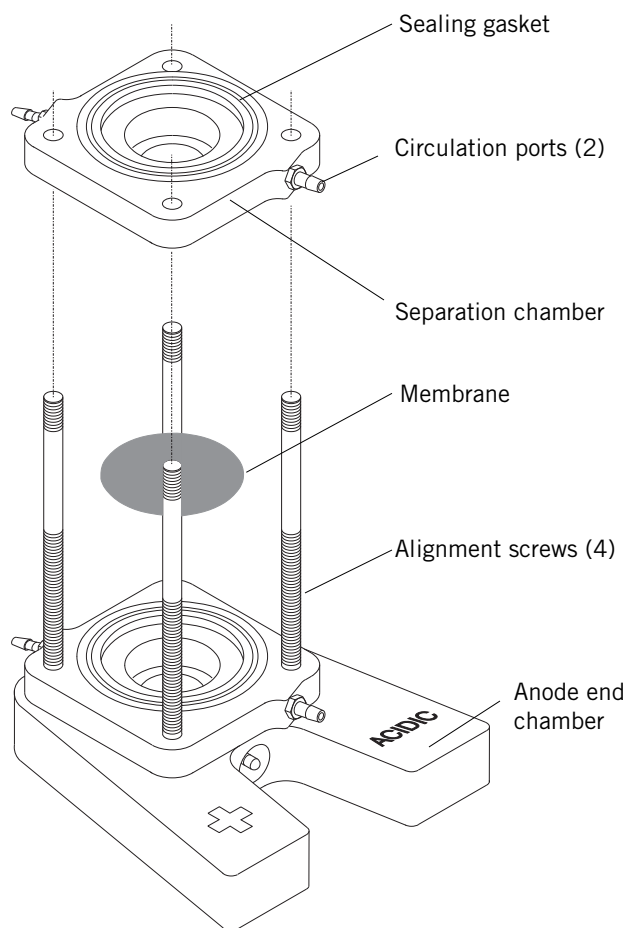
Refer to the assembly and installation section for instructions for assembling all cabinet components and calibrating the pump. Detailed separation module assembly instructions follow.

5.1 Pre-run preparation

Assemble the separation module

- 1 If the separation module is assembled, unscrew the four knobs and disassemble the module by sliding all stacked components off the metal screws.
- 2 Inspect each separation chamber for damage to the sealing O-ring gasket (replace if nicked or flattened) and check that both circulation ports are clean and screwed finger tight into each separation chamber.
- 3 Lay the anode (+) end of the assembly so that the 4 threaded alignment screws are facing upward and are ready to receive the separation chambers.
- 4 Arrange the washed membranes in sequence from the lowest pH (most acidic) to the highest pH (most basic). Following the indicator etched into the end piece, lay the most acidic membrane into the "acidic" end chamber. Orient the chamber so that the circulation ports are in the same position as the ports on the end piece. Lower the separation chamber onto the 4 screws and seat the chamber.
- 5 Carefully lay the next more basic membrane into the depression on the separation chamber and lower a second properly oriented separation chamber onto the four screws and seat it.

Figure 10.
Separation module assembly



- 6 Repeat step 5 until all membranes are installed in sequence. The last membrane should be the most basic.
- 7 After all separation chambers are in place, seat the cathode (-) end chamber onto the 4 screws and fit the 4 black knobs onto the screws, tightening them until the assembly is secure. (Finger tighten the knobs, making sure to tighten each one equally.)

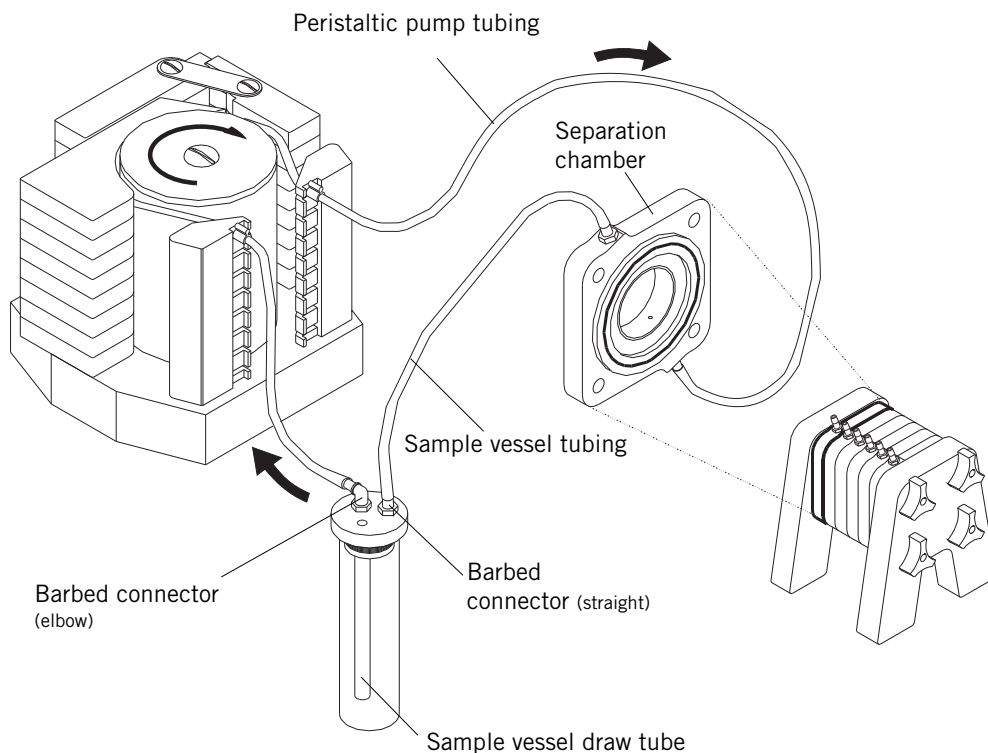
Create a fluid path for each chamber of the separation module

Each chamber used (between 1 and 8) requires a separate fluid circuit that runs from the sample vessel, through peristaltic pump tubing, into the lower chamber port, out of the upper chamber port, and back into the sample vessel. Install the path components systematically, keeping the tubing in order with the numbered tubing clips provided.

- 1 Install one length of peristaltic tubing for each chamber used according to the instructions in section 2.4 under the heading "Peristaltic tubing installation." Gather each set of tubing ends into a numbered clip at both the inlet end and the outlet end of the peristaltic tubing to help organize the tubing. Make sure each section of the path is labeled consistently.
- 2 Assemble the separation module according to instructions in the previous section.
- 3 Slide each peristaltic tubing outlet end (near the back of the unit) onto the lower port of each separation chamber, keeping them in the same order (1-8) as before.

Figure 11.
Fluid flow diagram

The protein is drawn through the sample vessel draw tube by peristaltic pump action on the peristaltic tubing. The sample then enters the lower port of the separation chamber and circulates back into the sample vessel by way of the sample vessel tubing.



- 4 Slide the right electrode post to the position denoted by the number of separation chambers to be used (3 – 8). Seat the separation module onto the electrode posts, with the positive end (marked +) on the left, and matching the black knobs to the black electrode post on the right.
- 5 Slide the intake end of the peristaltic tubing over the barbed elbow fitting on the sample vessel cap. (The elbow fitting extends to the draw tube.)
- 6 Connect the upper port of each separation chamber to the straight fitting on the sample bottle cap with a length of 3.0-mm sample vessel tubing.
- 7 After installation trace the path of each circuit to verify that the tubing connects the corresponding system components.

5.2 Prerun

Prerun the unit a minimum of 2 hours to verify that all system components are operational, to wash the unit with the buffer and solubilizer solution, and to test the membranes.

- 1 Turn on the IsoPrime mains power switch.
- 2 Turn on the pump mains power switch. If necessary, calibrate each channel of the pump. (Refer to p. 11, Adjusting fluid flow rate.) The pump flow rate should be between 3 to 4 ml per minute.
- 3 Fill each sample vessel with 30 ml of ddH₂O, ultra-pure water such as Milli-Q, or the solubilizing solution and lower a spin bar into it.
- 4 Turn on the pump and check the flow path for each sample vessel to make sure the fluid is flowing and following the intended path.
- 5 After all the chambers in the separation module are filled, lower the safety enclosure lid and plug the safety leads at the right side of the IsoPrime unit into the external power supply.

Note: DC power is supplied to the separation module only when the safety lid is closed.

- 6 Prerun the unit for a minimum of 2 hours at a constant power of 4 W. The current should start below 10 mA and gradually drop. The current should never exceed 25 mA if the solution is free of salt and contaminants.

The voltage should start at between 500 - 900 V and gradually climb to between 1500 - 3000 V or higher by the end of the run. Initial voltages can vary widely because the solubilizing agent and the number of chambers used affects the voltage required to maintain 4 W.

- 7 At the end of the prerun turn off the power supply, open the lid, and reverse the pump to drain the liquid in all chambers back into the sample vessels. Turn off the pump and empty all vessels.

Note

If the current exceeds 25 mA, the solution may contain a high salt concentration or other contaminant. Avoid high current because it can rupture the membranes.

5.3 Purification Run

Select electrodic buffers

Electrodic buffers for the anodic and cathodic chambers should be selected to provide just enough ions to create a current within the separation chamber. For most applications, dilute weak acids and bases (1-10 mM) or ddH₂O are suitable. Refer to the application notes for examples.

Table 4.
Electrodic buffers

Weak acids and bases that have been successfully used as electrodic buffers.

Acids

Glycine
Glutamic acid
Acetic acid
Citric acid
γ-amino butyric acid
Formic acid

Bases

CAPS
HEPES
Lysine
Arginine
Histidine

ddH₂O may be used for certain neutral or acidic proteins
NaOH (<2mM) can be used but may result in pH shock for certain proteins

Note

Electrodic solutions for proteins in the alkaline range should have pH values near the pH range in which fractionation takes place.

Adjust the pH of the dilute acid or base to within 1 to 2 pH units of the membrane pH at each electrode. Avoid titrating with strong acids and bases. Then add the same concentration of solubilizing agent used in the other sample vessels. Fill the sample vessel in the cathode chamber circuit with catholyte buffer and the sample vessel in the anode chamber circuit with anolyte buffer.

Load the sample

Small to medium quantities

If the IsoPrime is intended to separate moderate to heavily contaminated proteins, use one of two strategies to purify the sample:

Strategy A

Contaminants migrate out of the separation target chamber.

Fill all sample vessels with the solution used to solubilize the protein except the sample vessel that feeds into the chamber delimited by membranes with pH values slightly above and slightly below the pI of the target protein. Fill this remaining sample vessel with the protein solution. Under electrophoretic separation, contaminants (or other isoforms) will move out of the chamber containing the target protein and into adjacent chambers, leaving a purified product.

Strategy B

Target proteins migrate out of the contaminant chambers

Or, load the protein into one of the adjacent chambers delimited by membranes with pHs slightly above or slightly below the pI of the protein to be purified. Under electrophoretic separation, the protein of interest moves into another chamber, leaving contaminants behind. This method is particularly useful when the pI of the protein of interest is significantly different from all contaminants.

Worksheet 2. Purification run inventory

Date _____
 Starting sample ID _____
 Concentration _____
 Separation solution _____

Chamber	sample vessel contents <small>(distilled water, anolyte, catholyte, sample, contaminant, etc.)</small>	membrane pH	protein pI
(anode, +) 1	_____	_____	_____
2	_____	_____	_____
3	_____	_____	_____
4	_____	_____	_____
5	_____	_____	_____
6	_____	_____	_____
7	_____	_____	_____
8	_____	_____	_____

Power supply settings

	Prerun initial/final	Purification run initial/final
V	_____ (4000 V max)	_____ (4000 V max)
mA	_____ (25 mA max)	_____ (25 mA max)
W	_____ (4 W constant)	_____ (4 W constant)

Larger quantities

If the IsoPrime is to be used to purify relatively large volumes of proteins containing small quantities of impurities (such as pyrogens or endotoxins), the middle chambers (up to 6) can be combined to form one large chamber. Install membranes in only the anode and cathode chambers. For this application, use the large (125 ml) vessels, which fit into the middle of the sample vessel tray.

Purification run procedure

- 1 Turn on the pump and check the flow from the sample vessel to the inlet port to make sure the fluid is following the intended path and fills the separation module. To speed the filling process, press the RABBIT button on the pump.
- 2 Lower the safety lid, turn on the power supply, and set it to 4 W constant power. As in the pre-run, the initial current usually starts between 8 and 20 mA and eventually drops to 1 to 4 mA. The current should never exceed 25 mA, and if it begins to climb above 20 mA, evaluate the sample for contaminants such as salt.

The initial voltage should start between 300 and 700 V and climb to 1500 to 3000 V. Never allow the voltage to exceed 4000 V. Typically the voltage reaches its maximum within a few of hours and remains constant for the duration of the run. (Constant voltage does not indicate that the purification is complete.)

- 3 Periodically check the sample purity: remove a small aliquot (see below) from each sample vessel and analyze for the level of purity with such methods as IEF, HPCE, HPLC or SDS-PAGE. An IPG gel is the most discriminating method.

Sampling during the purification run

To sample during the run, turn off the power supply and open the safety enclosure lid. Remove aliquots through the sampling ports on the sample vessel caps, then close the lid and restart the power supply.

5.4 Protein Recovery

The purification process may take from 2 hours to 3 or more days, depending on the characteristics of the protein and the degree of resolution required. Resolution of proteins or isomers differing by less than 0.1 pI generally require a minimum of 8 hours.

- 1 At the end of the purification process, turn off and disconnect the power supply, open the safety lid, and reverse the direction of the pump by pressing the key with the arrow pointing counter-clockwise. Drain the chambers and the tubing completely back into the sample vessels.

Note: Electroendosmosis may cause liquid movement between chambers, and result in some vessels receiving more volume than others. If overflow is imminent, stop the pump and remove liquid from the overly full sample vessel and store it in a labeled container. (If the solution is coated with paraffin oil, draw the sample from the bottom of the vessel with a syringe or pipette.)

- 2 Transfer the solution from each vessel to a storage container.

At this point either restart the unit with the same membranes – if they are still usable and if the same protein will be purified – or disassemble the unit and clean it.

- 3 To reuse the membranes, fill the sample vessels and start the next run or wash the membranes with ddH₂O and store them in 20 mM sodium acetate (pH 5.0) and 2 mM sodium azide.
- 4 To disassemble the IsoPrime, lift the separation module from the posts and disconnect the tubing from the inlet and outlet ports on the separation unit. To clean the unit, see the Care and Maintenance section below.

6

Troubleshooting**Isoprime mains power***No power*

- ✓ Check that the power cord is plugged into a working receptacle.
- ✓ Examine fuses and replace if necessary.

Pump*Blank display*

- ✓ Check that the inside cabinet power cord is properly plugged into the pump power receptacle.
- ✓ Check that the mains power is plugged into a functioning power outlet.
- ✓ Check that both the pump and the mains power switches are on.
- ✓ Examine fuses and replace if necessary.
- ✓ If the display remains blank, the transformer or the fan assembly could be faulty. Contact Amersham Pharmacia Biotech.

Dead keypad or faulty pump head

Contact Amersham Pharmacia Biotech.

Membranes*Won't polymerize*

- ✓ Use only recent stock of the highest purity chemicals.
- ✓ Make 40% APS solution immediately before casting the membrane.
- ✓ Make sure the filters are completely saturated with no air pockets above or below.
- ✓ After casting, allow a full 20 minutes before moving the casting chamber and then heat the gel cassettes for 1 hr at 50 °C or leave overnight at room temperature.

Cratered surface

- ✓ Cover membrane with a glass cover plate and do not disturb for a full 20 minutes after casting.
- ✓ Make sure there are no air pockets above or below the filter.

Bowed or ruptured

- ✓ Slight bowing out of the membrane during a purification run is common, but extreme bowing (due to high current) damages the membrane. Refer to section 4.2 for information on desalting and decontaminating the sample in order to lower the current.
- ✓ If membranes were used for a previous run, check membrane integrity.

Prerun*Current >20 mA*

- ✓ Thoroughly clean all tubing and instrument components according to instructions in section 7.1. (Do not use any ionic detergents.)

Purification run*Current >20 mA*

- ✓ Desalt sample so that less than 50 mM of salt remains. Do not expose proteins or instrument to ionic detergents such as SDS. (Refer to section 4.2)

Electroendosmosis

- ✓ If a large volume shift occurs, the separation may be complete; analyze the sample.
- ✓ If the run proves to be incomplete, reduce the protein concentration and load the sample into the sample vessel connected to the chamber into which the proteins are meant to migrate.
- ✓ If it is necessary to repeat the run, completely desalt the sample first.

Sample precipitated

- ✓ Add a solubilizing agent or increase its concentration and use it in the same concentration throughout the entire procedure.
- ✓ Prepurify the sample to 50 - 70% purity to prevent non-specific aggregation.
- ✓ Lower sample concentration.

pI shifted

- ✓ Shorten run time. Long runs may denature or degrade the protein and change its characteristics.
- ✓ Determine the pI of the protein under the same conditions encountered during the run: use the same concentration of solubilizing agents and maintain the same temperature. (Refer to section 4.4)
- ✓ Add reducing agents such as β -mercaptoethanol to prevent disulfide bond formation.

Special cases*No protein separation*

- ✓ Trace all tubing connections to make sure that each fluid path connects the correct chamber and sample vessel. See section 5.1 for prerun set up.

*Alkaline samples
(pH>8)*

- ✓ Avoid using urea as a solubilizing agent to prevent carbamylation of the protein.

Oxidized proteins

- ✓ At acidic pH values only, prevent proteins from oxidizing by adding β -mercaptoethanol.

*Large proteins
(200-300 kD)*

- ✓ If proteins do not cross membranes because the protein is too large, decrease the %T to 5% and increase the %C to 8%.

7 Care and Maintenance

7.1 Cleaning

Cabinet components

Switch the mains power off and unplug the power cord before cleaning the unit. Wipe all surfaces with a soft damp cloth to remove residues and then dry with a towel.

Clean the separation unit, sample vessels, and caps with standard laboratory detergents such as RBS or Alconox. Solvents are not recommended. Never use abrasive cleaners. After cleaning, rinse thoroughly with ddH₂O.

Tubing

Simple rinse

Simply flushing the entire tubing system with distilled water or separation solution may be sufficient between identical runs if the protein does not show evidence of aggregation, precipitation or "stickiness." Submerge the inlet ends of the pump tubing in a beaker containing at least 250 ml of water or separation solution. Pump the solution into another beaker at normal pump speed. Use a syringe to flush the tubing pieces that connect the separation chambers to the sample vessels.

Complete wash

Flush tubing thoroughly with 8 M urea as described above if there is evidence of protein adsorption or if the unit is to be used for different proteins. After completing the urea wash, flush the tubing with distilled water two or three times.

Replace any tubing observed to be severely fouled or worn.

7.2 Repair

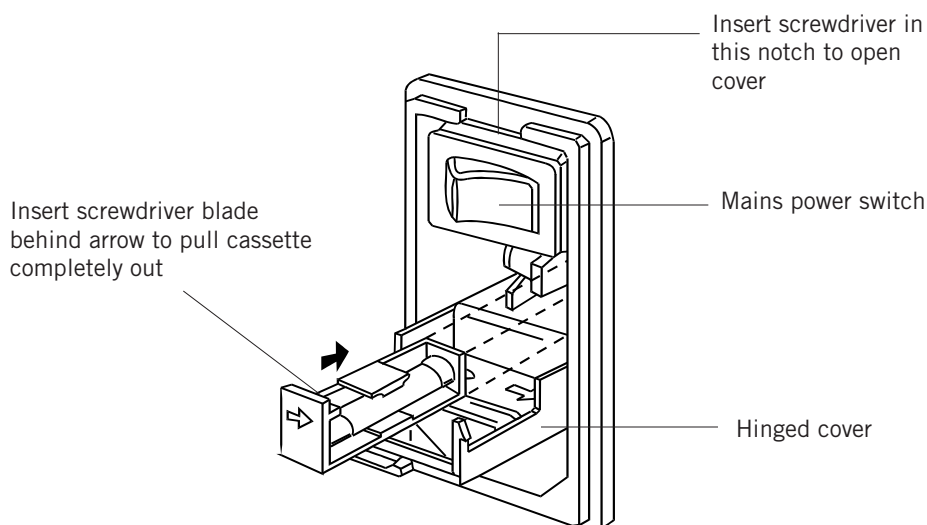
If the IsoPrime malfunctions, call an Amersham Pharmacia Biotech technical service representative to evaluate the problem. Your instrument may still be under warranty, or you may wish to arrange to send it in for repair after the warranty period has expired. A service manual (code no. 80-6438-15) is available.

7.3 Replacing fuses - IsoPrime mains power module

The two input fuses in the mains power module in the back of the unit do not require maintenance, but one or both may fail due to fatigue under normal operating conditions. Fuses protect equipment by disconnecting loads too large for the instrument's circuit design, so it is imperative that fuses are replaced only by identical fuse types (F 1A/250V 5x20mm). Figure 12 illustrates how to replace the mains power module fuses. (To replace pump fuses, refer to section 2.4.)

Figure 12.
IsoPrime mains power module

The power module is located at the back of the IsoPrime cabinet.



- 1 **Caution:** Turn the mains power supply switch off and detach the power cord before replacing input fuses!
- 2 Open the fuse compartment by inserting a small flat-blade screwdriver into the slot at the left side of the power module. Twist the screwdriver 1/8-turn to release the cover, then pull out the hinged compartment, which opens to the right.
- 3 Insert the screwdriver above the arrow on one fuse cassette, catch the cassette end, and slowly slide it completely out of the module.
- 4 Pull the fuse out of its cassette and inspect. If the fuse element is burned or broken, replace the fuse with an identical type. If the fuse appears to be intact, check it with an ohmmeter. (A short circuit indicates the fuse is still usable.)
- 5 After placing a good fuse into the cassette, slide it into the power module, making sure the arrow on the cassette points to the right (in the same direction as the guide arrows on the inside of the compartment door.)
- 6 Repeat steps 3 – 5 for second cassette.
- 7 Close the fuse compartment cover and gently press it into the power module until it snaps shut.
- 8 Plug unit in and turn mains power switch on.

A

Specifications

Power

IsoPrime AC mains power

115 V model 115 VAC, 50/60 Hz, 70W
 230 V model 230 VAC, 50/60 Hz, 70W
 2 fuses: F 1A/250V 5x20mm

External HV DC power supply

Maximum rating: 4000 V, 50 mA, 15 W

(Powers the separation module)

Pump

Line Voltage 90 V to 132 V for 110 V model
 180 V to 264 V for 220 V model
 Line Frequency 47 to 63 Hz
 Fuse values 115 V - two 0.5 A/250 V, timed, (6.3 x 32 mm)
 230 V - two 0.25 A/250 V, timed, (6.3 x 32 mm)
 Power consumption Full load: 45 W at 220 V ; no load: 35 W at 220V
 Temperature Operating temp: 0-40 °C. No warm-up time is required.
 Humidity Up to 95%, non-condensing
 Position Upright only (vertical ± 5°)
 Head speed 0.01 to 48 rpm
 Torque Greater than 3 Nm at any speed below 25 rpm, at or above
 the nominal voltage (110V or 220V)
 Motor speed stability 0.5% for any variation of line voltages torque or temperature.
 Continuous speed adjustment From 0-48 rpm by 0.01 increments up to 9.99 rpm, above
 9.99 rpm by 0.1 increments
 Flow rate range Maximum: 26 ml/min (2.8 mm tubing)
 Maximum back pressure 500 kPa

IsoPrime dimensions

Dimensions 51 x 51 x 38 cm (20 x 20 x 15 in)
 Shipping volume 0.24 m³ (8.66 ft³)

Weight

Net (includes pump) 26 kg (57 lbs)
 Shipping (total) 37 kg (81 lbs)

Environmental conditions

Altitude to 2,000 m
 Temperature 5 - 40 °C
 Maximum relative humidity ≤80% for temperatures 5-31 °C, decreasing linearly to 50%
 relative humidity from 31-40 °C.

Safety Specifications

Built to UL 1262 and IEC 1010-1 safety standards and listed by ETL Testing Laboratories.

C

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Development of PrIME Technology

(Preparative Isoelectric Membrane Electrophoresis)

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D

Purification Protocol

Carbonic Anhydrase (bovine erythrocytes)

This basic protocol can be used to check instrument performance or to become acquainted with the instrument and preparative isoelectric membrane electrophoresis (PrIME) techniques.

Sample characteristics

The protein for this protocol, bovine erythrocyte carbonic anhydrase (Sigma Chemical Co., catalog no. C-3934), is supplied at a purity greater than 90% as analyzed by SDS-PAGE. IEF analysis of samples reveals five major and several minor isoforms ranging in pI values from about 4.9 to 6.7. These isoforms can be fractionated into nearly pure species as shown by the results below.

Protein lots may vary, and measured pI values depend somewhat on the IEF method used. In our experience the pI of the most prominent band can range from 6.15 to 6.29. In the sample result below the pI value of this band is 6.15.

Purification results.

Original sample and sample vessel contents.

Gel

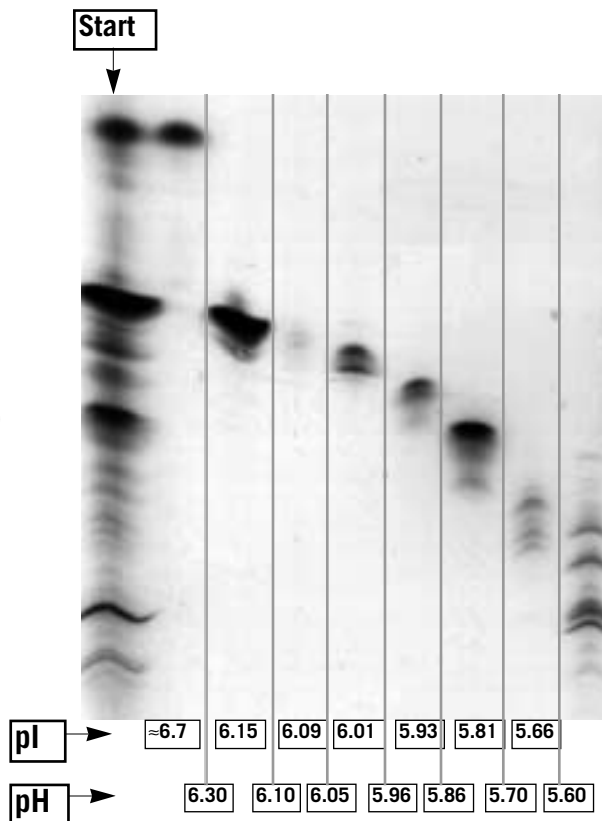
Isogel® carrier ampholyte IEF agarose gel, pH 3 -7 (FMC)

Protein

Carbonic anhydrase, bovine erythrocytes (Sigma Chemical Co., catalog no. C-3934)

Run conditions

3000 V max
20 mA max
4 W constant



Prepare the instrument

Note

Refer to the main instructions for more detail.

- 1 **Assemble the IsoPrime Multi-Chambered Electrofocusing Unit** in a 4 °C environment such as a cold room or large refrigerator.
- 2 **Prepare a set of membranes.** Worksheet 1 on the next page lists each membrane pH. Use positive displacement pipettes or a microsyringe to measure acrylamido buffers; accurate pipetting is critical for reproducible results. Wash membranes after polymerization is complete.
- 3 **Assemble the separation module.** Start by placing the most acidic membrane at the anode end chamber (labeled “acidic”) and assembling each chamber in order of increasing pH.
- 4 **Fill each sample vessel with 30 ml distilled water and set prerun parameters.**
 Peristaltic pump flow rate 6.5 rpm. Press clockwise arrow to start pump.
 Power supply 4 W constant, 20 mA max, 3000 V max
- 5 **Pre-run for 2 hours.** By the end of the prerun, the voltage should reach nearly 3000 V if no contaminants are present. (See the Troubleshooting section if contaminants are present.)

After two hours, drain the separation chambers into the sample vessels by reversing the pumping direction.

The purification run

Note

The protein concentration can be as low as 3 mg/ml. Clearer results are possible with the recommended concentration, however.

- 1 Prepare carbonic anhydrase solution. Dissolve 180 mg carbonic anhydrase in 30 ml of double distilled water. Filter with a low binding protein filter such as Millex GV4, 0.22 µm. Cool to 4 °C.
- 2 Replace the water in chamber 7 with 29 ml of the carbonic anhydrase solution. (The pH values of membranes delimiting this chamber are 6.10 and 6.30). Store the remaining 1 ml of solution for post-run analysis.
- 3 **Start the purification.** Fill the chambers by pressing the clockwise arrow on the pump keypad. Set the power supply to 4 W constant, 20 mA max., 3000 V max.
- 4 Continue the separation for a minimum of 12 hours. Longer runs for up to several days have no detrimental effects on the protein and allow more time for separation.
- 5 Drain the separation chambers into the sample vessels by reversing the pumping direction. Remove the purified sample.

Note

If irregularities occur during the run, consult the Troubleshooting section for possible causes.

Worksheet 1. Protocol for preparing 2 membranes at each pH used in the purification of carbonic anhydrase (bovine erythrocytes)

- A Convert Doctor pH concentrations (mM) into volume (μ l) of acrylamido buffer for 10 ml of solution: **Multiply conc. by 50.**
(Multiplier based on using 200 mM acrylamido buffers to make 10 ml of membrane solution.)

Membrane	1		2		3		4		5		6		7	
Membrane pH (calculated)	5.60		5.70		5.86		5.96		6.05		6.10		6.30	
	conc mM	vol μ l	conc	vol	conc	vol	conc	vol	conc	vol	conc	vol	conc	vol
pK 1.0	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 3.1	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 3.6	0.000	000	0.173	9	0.441	22	0.612	31	0.767	38	0.851	42	1.195	60
pK 4.6	4.740	237	4.268	213	3.524	176	3.048	152	2.617	131	2.383	119	1.429	71
pK 6.2	5.239	262	5.284	264	5.355	268	5.400	270	5.441	272	5.463	273	5.554	278
pK 7.0	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 8.5	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 9.3	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK 10.3	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
pK >12	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____

B	Fill ddH ₂ O to 2.000 ml	2.000 ml	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
C	Add acrylamido buffer vol.													
D	Fill ddH ₂ O to 6.000 ml, mix	6.000 ml	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000	6.000
E	Measure solution pH	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
F	Adjust pH to 6.5, \pm 0.2 with 1M Tris base (μ l), mix	6.5 30.0	6.5 23.0	6.5 19.0	6.5 16.0	6.5 13.0	6.5 10.0	6.5 6.0	6.5 6.0	6.5 6.0	6.5 6.0	6.5 6.0	6.5 6.0	6.5 6.0

Important: For the following steps, prepare only 1 membrane solution at a time and pour into mould before starting the next solution.

	%T/%C	10/8	10/8	10/8	10/8	10/8	10/8	10/8	10/8	10/8	10/8	10/8	10/8	10/8
G	Add acrylamide/bis stock (30%T)	<u>3333</u> μ l	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>	<u>3333</u>
H	Fill ddH ₂ O to 10.00 ml, mix	10.00 ml	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
I	Add TEMED (μ l)	5 μ l	5	5	5	5	5	5	5	5	5	5	5	5
J	Add APS, 40% (w/v) (μ l), mix	10 μ l	10	10	10	10	10	10	10	10	10	10	10	10

Pour into mould. Do not disturb for 20 minutes.
Polymerize for 1 hr at 50°C or overnight at room temperature.
Disassemble and start washing procedure.

Worksheet 2. Purification run inventory

Date _____
 Starting sample ID Carbonic anhydrase (bovine erythrocytes)
 Concentration 6 mg/ml (180 mg/30 ml)
 Separation solution dd H₂O

Chamber	sample vessel contents <small>(distilled water, anolyte, catholyte, sample, contaminant, etc.)</small>	membrane pH	protein pI
1 (anode, +)	<u>30 ml dd H₂O</u>	<u>5.60</u>	<u>5.66</u>
2	<u>30 ml dd H₂O</u>	<u>5.70</u>	<u>5.81</u>
3	<u>30 ml dd H₂O</u>	<u>5.86</u>	<u>5.93</u>
4	<u>30 ml dd H₂O</u>	<u>5.96</u>	<u>6.01</u>
5	<u>30 ml dd H₂O</u>	<u>6.05</u>	<u>6.09</u>
6	<u>30 ml dd H₂O</u>	<u>6.10</u>	<u>6.15</u>
7	<u>29 ml protein solution</u>	<u>6.30</u>	
8 (cathode, -)	<u>30 ml dd H₂O</u>		

Power supply settings

	Prerun initial/final	Purification run initial/final
V	<u>1700/2700</u> <small>(4000 V max)</small>	<u>500/2988</u> <small>(4000 V max)</small>
mA	<u>5/2</u> <small>(25 mA max)</small>	<u>5/2</u> <small>(25 mA max)</small>
W	<u>4/4</u> <small>(4 W constant)</small>	<u>4/4</u> <small>(4 W constant)</small>

Analyze the results

Use either an Immobiline DryPlate or a carrier ampholyte agarose IEF gel, pH 3 – 7, to analyze sample vessel contents. An Immobiline gel generally determines pI values more accurately, but a carrier ampholyte gel is usually easier to handle, and is therefore useful in monitoring progress. Results from the two different gel types may not correlate well.

Immobiline DryPlate, pH 4 – 7. Follow instructions accompanying the gel to hydrate and prepare the gel. Load 10 – 20 µl per lane. Include lanes of the original sample and contents of each sample vessel for comparison. Run for about 2 hours at 3000 V. Stain and destain the gel.

Carrier ampholyte IEF agarose gel, pH 3 – 7. Follow instructions accompanying the gel. Load 10 – 20 µl per lane. Include lanes of the original sample and contents of each sample vessel for comparison. Run for about 1 hour at 1000 V. Stain and destain the gel.

Suggested chemicals and materials

Chemicals	*Catalog Number
Carbonic anhydrase (Bovine erythrocyte, Sigma)	#C-3934
Acrylamide IEF (Electrophoresis grade)	17-1301-01
Bisacrylamide	17-1306-01
Ammonium persulfate	17-1311-01
TEMED	17-1312-01
Repel-Silane, ES	17-1332-01
Immobiline II Acrylamido buffers	
pK 3.6	80-1255-70
pK 4.6	80-1255-71
pK 6.2	80-1255-72
Materials	
Low protein binding filters, .22 µm	Millipore
Immobiline DryPlates, pH 4-7	80-1128-28
Roller (for plates)	80-1106-79
IEF Electrode strips	18-1004-40h
Reswelling cassette and clamps	18-1013-74
Positive displacement pipettes	Rainin
Hamilton syringe	Fisher
Multiphor II	18-1018-06
EPS 350IXL (HV Power supply)	18-1130-05
* from Amersham Pharmacia Biotech, unless otherwise noted	

E

Warranty Statements

Important user information

Please read this entire manual to fully understand the safe and effective use of this product.

English



The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument.



The lightning symbol within an equilateral triangle is intended to alert the user to the risk of exposure to high voltages.

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Pour une bonne compréhension et une utilisation en sécurité maximale, il convient de lire entièrement ce manuel.



Dans la documentation qui accompagne l'instrument un point d'exclamation dans un triangle équilatéral a pour but d'attirer l'attention de l'utilisateur sur des instructions importantes de fonctionnement ou de maintenance.



Le symbole de l'éclair dans un triangle équilatéral a pour objet d'attirer l'attention de l'utilisateur sur un danger d'exposition à la haute tension.

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Información importante para el usuario

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Español

El signo de admiración en un triángulo equilátero en el manual, advierte al usuario sobre la presencia de instrucciones importantes de operación y mantenimiento del aparato.



El símbolo del rayo en un triángulo equilátero alerta al usuario sobre el riesgo de exposición a altas tensiones.

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Italiano

Il punto esclamativo all'interno di un triangolo equilatero indica all'operatore la presenza di importanti istruzioni di funzionamento e manutenzione nella documentazione allegata al prodotto.



Il simbolo del fulmine all'interno di un triangolo equilatero indica all'utente la presenza di un rischio di esposizione ad alte tensioni.

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Ordering Information

	Qty	Code No.
IsoPrime Multi-chambered Electrofocusing Unit. Includes 8-channel peristaltic pump, separation module, and Doctor pH software.		
115 VAC	1	80-6081-90
230 VAC.	1	80-6082-47
Glass sample vessels, 30 ml.	12	80-6082-09
Glass sample vessel covers, 30 ml	1	80-6082-28
Sample vessel rack, 30 ml	1	80-6082-66
Stir bars, 0.75 in Star	4	80-6082-85
Peristaltic tubing, 2.79 mm ID	8	80-6083-04
Sample vessel tubing, 3 mm ID	8	80-6083-23
Numbered tubing clips	3	80-6083-42
Filters, GF/D, 4.7 cm	100	80-6083-61
Membrane casting chamber, dual	4	80-6083-80
Membrane casting chamber cover glass	8	80-6083-99
Power cord, 115 V	1	80-6106-03
Power cord, 230 V	1	80-6230-10
PI 8 Instruction manual	1	80-6397-11
PI 8 Service manual	1	80-6438-15
Doctor pH instruction manual	1	80-6397-30
HV lead safety adapter (2 to 4 mm)	2	80-6105-46
HV lead safety adapter (2 to 4 mm recessed output)	2	80-6105-84
IsoPrime cabinet fuses, F 1A/250 V, 5x20 mm	5	80-6109-45
Pump fuses, 115 V - (0.5A-SB-3AG)	5	80-6107-93
Pump fuses, 230 V - (0.25A-SB-3AG)	5	80-6106-79
Acrylamide IEF	250 g	17-1300-01
	1 kg	17-1302-02
Acrylamide IEF 40% solution	1 litre	17-1301-01
ReadyMix IEF, to make ~120 ml 30% T, 3% C	41.5 g	17-1309-01
ReadySol IEF 40% T, 3% C	1 litre	17-1310-01
N-N'-Methylene-bisacrylamide	25 g	17-1304-01
	100 g	17-1304-02
N-N'-Methylene-bisacrylamide, 2% solution	1 litre	17-1306-01
Urea	500 g	17-1319-01
Glycerol, 87%	1 litre	17-1325-01
Triton X-100	500 ml	17-1315-01
CHAPS	1 g	17-1314-01
Repel-Silane, ES	500 ml	17-1332-01
Amberlite IRN-150L	500 g	17-1326-01

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