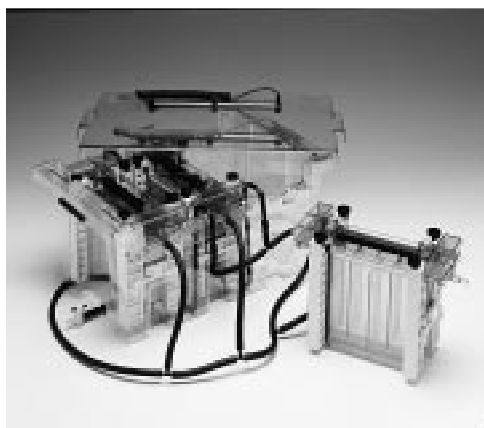

PROTEAN II xi multi-cell

Instruction Manual

Catalog Number 165-1951

BIO-RAD

Note

To insure best performance from the PROTEAN II xi multi-cell, become fully acquainted with these operating instructions before using the cell to separate samples. Bio-Rad recommends that you 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 PROTEAN II xi multi-cell components and accessories be cleaned with a suitable laboratory cleaner (such as Bio-Rad Cleaning Concentrate, catalog number 161-0722) and rinsed thoroughly with distilled water, before use.

Model ______________________________

Catalog No. ______________________________

Date of Delivery ______________________________

Warranty Period ______________________________

Serial No. ______________________________

Invoice No. ______________________________

Purchase Order No. ______________________________

Warranty

Bio-Rad Laboratories warrants the PROTEAN II xi multi-cell 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.

This warranty does not apply to parts listed below:

1. Platinum wire, glass plates.

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
General Information

1.1 Introduction

Two-dimensional electrophoresis can provide exceptionally high resolution of the protein components in a complex sample. It is capable of resolving several thousand individual protein species. The first dimension involves isoelectric focusing (IEF), where proteins are separated according to their isoelectric points. The second dimension resolves proteins on the basis of their molecular size. This protocol is based on the method of Hochstrasser et al.,[1] which uses higher power settings and more efficient cooling (and other modifications) than traditional 2-D protocols to produce higher resolution two-dimensional gels in less time.[1-3]

The PROTEAN II xi multi-cell is an advanced electrophoresis unit dedicated to producing extremely high quality two-dimensional polyacrylamide gels. The PROTEAN II xi multi-cell can be easily converted for 2-D electrophoresis using an optional conversion kit.

1.2 PROTEAN II xi multi-cell Specifications and 2-D conversion kit

Construction:	
Cooling core	Molded polysulfone
Lid and lower buffer chamber	Molded polycarbonate
Clamps, and cams	Glass and BF87Žfilled molded polycarbonate
Cooling coils	Aluminum, coated with electrically non-conductive material (PROTEAN II xi multi-cell 2D conversion kit only)
Electrical leads	Flexible, coiled
Electrodes	Platinum,0.010 inch diameter (0.254 mm)
Shipping weight	18 kg
Overall size	60 x 36 x 30 cm
Gel size	16 x 20 cm slab
Glass plate sizes	20 cm cell: 20 x 20 cm (inner plate) 22.3 x 20 cm (outer plate)
Cooling core, maximum flow rate	2 L/min
Voltage limit	1,000 VDC
Temperature limit	50 °C

Note: PROTEAN II xi multi-cell components are not compatible with chlorinated hydrocarbons (e.g., chloroform), aromatic hydrocarbons (e.g., toluene, benzene), or acetone. Use of such organic solvents voids all warranties.

1.3 Safety

Power to the PROTEAN II xi multi-cell is to be supplied by an external DC voltage power supply. This power supply musts 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:

1000 VDC ⎓	maximum voltage limit
80 Watts	maximum power limit
50 °C	maximum ambient temperature limit
50 °C	maximum temperature limit of liquid circulating through cooling core

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.

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

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

Section 2
Description of Major Parts

Central Cooling Cores

The central cooling cores house the upper and lower electrodes, and are the primary cooling units in the PROTEAN II xi multi-cell. They function to prevent thermal band distortion during electrophoretic separations. The serpentine flow pattern within the core assures even heat distribution over the entire gel area. An ethylene glycol:water (20:80) solution is recommended as coolant. Other coolants may damage the plastic. The temperature limit for the cooling core is a maximum of 50 °C.

Cooling Coils (PROTEAN II xi multi-cell 2-D conversion kit only)

The two cooling coils provided with the PROTEAN II xi multi-cell 2-D conversion kit chill the lower chamber buffer, which completely covers the electrophoretic gel. Both the cooling cores and the cooling coils must be connected to a refrigerated water recirculator. If a refrigerated water recirculator is not available, the cores and cooling coils can be connected to a tap water line; however the achievable resolution may decrease.

Sandwich Clamps

The unique PROTEAN II xi multi-cell sandwich clamps feature a single screw mechanism which makes assembly, alignment, and disassembly of the gel sandwich effortless. The clamps exert an even pressure over the entire length of the glass plates, providing a leakproof seal and preventing plate damage due to uneven pressure. Each pair of clamps consists of a left and a right clamp. The sandwich clamps can accommodate up to two 1.5 mm thick gels using the notched inner glass plates.

Upper Buffer Chamber

The completed gel sandwich attaches to the central cooling core, so that the outer plate of the sandwich forms the side of the upper buffer chamber. The inner plate is clamped against a rubber gasket on the central cooling core to provide a greaseless, leak-free seal for the upper buffer. The long plate of each sandwich forms one side of the cathode chamber. The optional tube gel adaptors also snap onto the central cooling core to form the upper buffer chamber walls (one adaptor per side). If only one gel is to be run, an upper buffer dam is attached to the core to form the complete upper buffer chamber. Each upper buffer chamber will hold approximately 350 ml of buffer when full.

Lower Buffer Chamber

The lower buffer chamber of the PROTEAN II xi multi-cell encloses the unit and provides stability during electrophoresis. The molded unit requires approximately 20 liters of buffer to completely cover the gels or a minimum of 5 liters to perform SDS-PAGE. With 2-D gels, the cooling coils are immersed in the lower running buffer, providing optimal heat dissipation. Do not overfill. See section 3.5, paragraph 2.

Lid

Combined with the lower electrophoresis chamber, the lid acts to fully enclose the PROTEAN II xi multi-cell during electrophoresis, thus providing electrical isolation. The lid cannot be removed without disconnecting the electrical circuit. It can be placed on the lower chamber in only one orientation, so that the anode and cathode connections cannot be accidentally reversed.

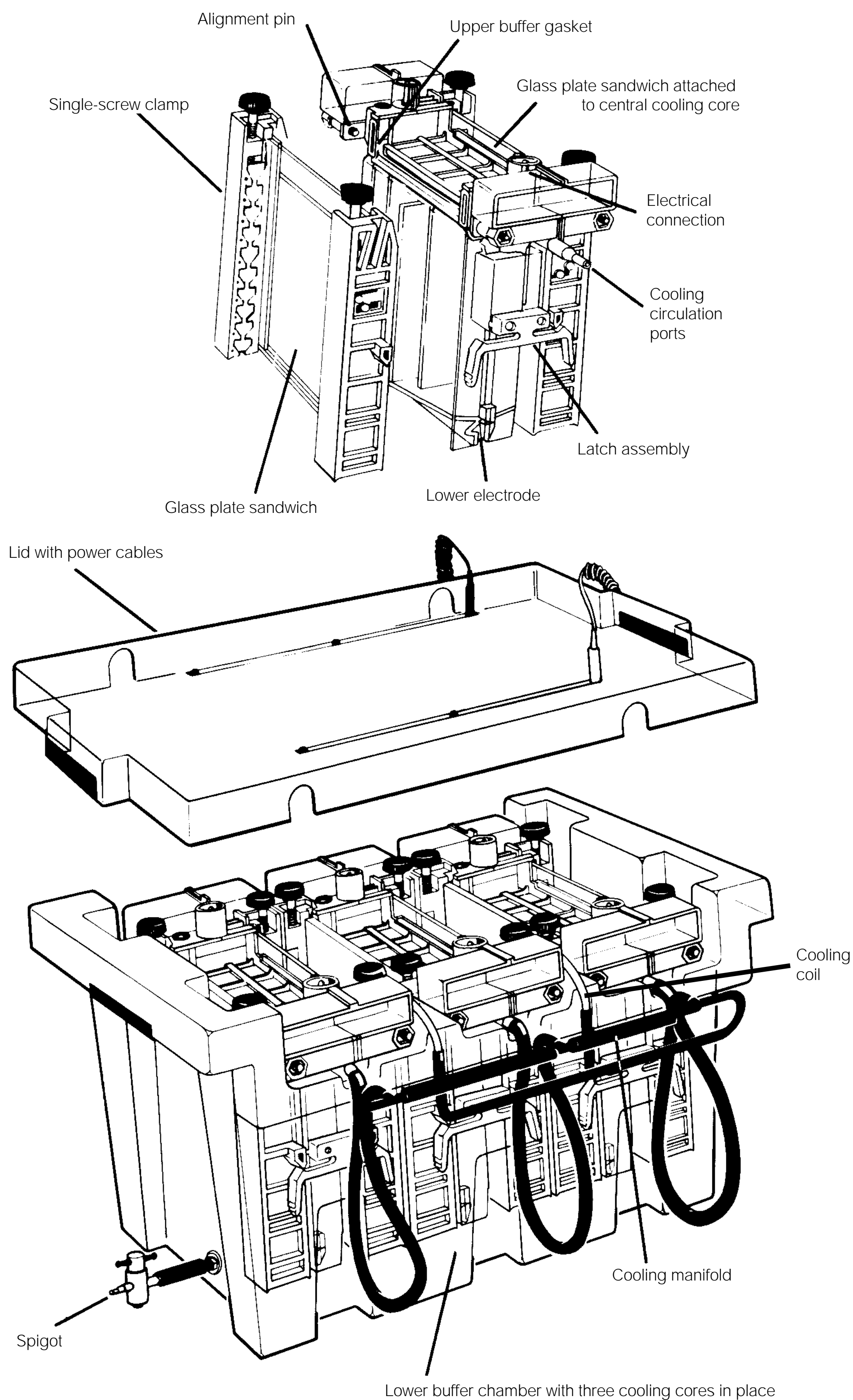

Fig. 1. Parts of the PROTEAN II xi multi-cell with PROTEAN II xi multi-cell 2-D conversion kit.

Section 3
Two-Dimensional Electrophoresis

The PROTEAN II xi multi-cell is primarily intended for the second dimension of 2-D electrophoresis. While it is also possible to run the first dimension tube gels in the multi-cell using the PROTEAN II xi tube gel adaptor, it is more practical to run the first dimension in a smaller cell such as the Model 175 Tube Cell (catalog number 165-1980). The method of running the first dimension gels should be determined before beginning the following protocol, which includes both the first and second dimension of 2-D. Since the PROTEAN II xi multi-gel casting chamber allows you to cast slab gels up to 1 week prior to use, you may wish to cast the resolving portion of the slab gels before beginning the 2-D protocol below. Use of the PROTEAN II xi multi-gel casting chamber is detailed in separate instructions.

The chart below outlines in sequence the essential steps of 2-D electrophoresis, and refers to the protocols in Section 7. Since these protocols do not require the use of a stacking gel, the resolving gels may be cast up to a week in advance, or they may be cast while the first dimension tubes are running and stored overnight.

3.1 Sequence of Steps for 2-D Protocol

Step	Time Interval
DAY 1	
1. Pour tube gels	polymerize 2 hours
2. Prepare chamber electrolytes, prepare and load samples	½ hour - 1 hour
3. Electrophorese at 200 V constant voltage	2 hours
4. Electrophorese at 500 V constant voltage	2 hours
5. Electrophorese at 800 V constant voltage	16 hours (overnight)
6. Cast slab gels for second dimension gels while first dimension gels are running	1 hour
7. Prepare second dimension running buffer	10 minutes
DAY 2	
8. Disassemble tube apparatus	2 minutes
9. Extrude gels from tubes and overlay tube gels onto slab gels	25 minutes
10. Electrophorese the second-dimension SDS gel	4-4½ hours
11. Stain and analyze results	

3.2 Protocol for IEF First Dimension

Casting IEF Tube Gels

For reproducible 2-D gels, it is essential that the IEF tube gels be precisely the same length and that polymerization be identical from day to day. Care must be taken in pouring the gels to the same height so that the polymerization height will be the same from tube to tube. An overlay step is not necessary in IEF first dimension tube gels. The meniscus formed on top of the gel will not influence the pH gradient or the resolution of the bands. The advantage of not overlaying is the formation of gels of more uniform length and composition. Stock solutions and formulations for first dimension tube gels are given in Section 7.

1. Mark the capillary tubes (1.5 mm ID, 7.5 mm OD, 180 mm, Bio-Rad catalog number 165-3138) with a laboratory marker 14.0 cm from one end.
2. Connect each capillary tube to a 1 ml syringe using a small piece of Tygon tubing 3⁄16" ID x ¼" OD, and approximately 2 cm in length (not included). Fill either a test tube rack or a level casting stand, such as Bio-Rad's Model 225 Tube Gel Casting Stand (catalog number 165-2020) with a disposable 12 x 75 mm test tube for each capillary tube. Insert a capillary tube/syringe assembly into each test tube.
3. Prepare the first dimension monomer solution and degas well. (The removal of molecular oxygen by degassing is essential for reproducible polymerization.)

   Warning: Always wear gloves to prevent exposure to acrylamide.
4. Add the APS and TEMED, and swirl 8 to 10 times. Working quickly, pipet 1 ml of acrylamide solution into each test tube. Using the syringe, pull up the liquid in each tube to the 14.0 cm mark. Let the capillary tubes sit undisturbed, with syringes attached, for 2 hours at room temperature to allow complete polymerization to occur.
5. After polymerization is completed, remove the capillary tubes from each test tube. Remove the syringe and Tygon tubing. Press and rotate the bottom of the capillary tube squarely down on a piece of Parafilm to remove excess acrylamide. Wipe off the excess acrylamide.
6. Inspect the gels before loading in the isoelectric focusing apparatus. Bubbles within the gel prevent focusing, and these gels should be discarded.

   Note: Alternative methods for filling capillary tubes can be used, such as wrapping the bottom end of the capillary tube with two layers of Parafilm™ laboratory film and filling the tubes using a syringe and fine gauge cannula (gel tube loading needle, 165-1943). The cannula should be long enough to reach the bottom of the tube. Slowly inject the acrylamide solution into the bottom of the tube, withdrawing the cannula as the acrylamide enters the tube and fill to the mark on the tube.

Sample Preparation and Loading

Sample preparation prior to isoelectric focusing is one of the most important steps for obtaining reproducible two-dimensional electrophoresis gels. There is no method which is optimal for every sample, and it may be necessary to experiment with different protein solubilization methods to determine which is best.

1. Prepare the first dimension running electrolytes as described in Section 7.
2. Prepare the IEF sample concentrate solution A and/or iso-urea solution E (Section 7). These solutions should be prepared fresh, or frozen in aliquots.

   Note: Sample loads above 400 µg total protein may cause loss of resolution in the second dimension slab gel.
3. Prepare the sample just before loading. The amount of denaturing sample solution A and/or iso-urea solution E will depend upon the protein concentration of sample and upon the type of sample. An initial ratio of 1 µl IEF sample concentrate for every 10 µl sample can be used. For denaturing, samples are heated at 95 °C for 5 minutes then cooled for 2 minutes at room temperature ***before*** loading or before adding iso-urea solution. Review references 1 and 2 by Hochstrasser et al. for additional information on sample treatment.
4. Pour lower electrolyte (10 mM H_3PO_4) into the lower chamber. Load the tubes into the tube gel apparatus. Plug any unused tube holes with stoppers.

5. Load the samples with a Hamilton syringe, or with a Drummond pipet tip. (Generally, 15 to 30 µl of final diluted sample is loaded.)
6. Carefully overlay the sample with upper electrolyte (20 mM NaOH) without mixing sample with upper buffer, then completely fill the tank upper reservoir with the upper electrolyte.

Running the IEF Gels

1. Place the lid on the focusing unit, and attach the power cables to an appropriate power supply (see Section 9.6).
2. Run the first dimension gels at room temperature with a constant voltage of 200 volts for 2 hours, followed by 500 volts for 2 hours, and then 800 volts overnight (16 hours).

3.3 Protocol for Casting Resolving Gels

1. Follow the PROTEAN II xi multi-gel casting chamber manual or detailed instructions on casting chamber preparation. Prepare the chamber using 1.5 mm spacers and beveled inner glass plates, catalog numbers 165-1827 (16 x 20 cm) or 165-1828 (20 x 20 cm). Beveled inner glass plates facilitate the application of the 1st dimension gel to the slab gel.
2. After the casting chamber is prepared, each gel can be tagged with identifying information. Use 1 cm^2 pieces of Whatman filter paper which have a series of numbers typed on them, or written in ink. Drop the pieces into the glass plate sandwiches near a corner. The paper will be saturated with monomer solution and doesn't interfere with the running of the gel.
3. Prepare a 1 ml syringe for each gel by filling it with water-saturated sec-butanol. Attach a small pipet tip to each syringe. These syringes will be used to overlay the resolving gels as discussed in the multi-gel casting chamber manual.
4. Prepare the second dimension resolving gel solution as described in Section 7.

   Warning: Always wear gloves to prevent exposure to acrylamide.
5. Attach a piece of Tygon tubing (~10 cm) to the luer fitting port on the chamber. Attach a three-way stopcock to the tubing and a 50 ml syringe to the stopcock. Attach another length of Tygon tubing to the remaining opening of the stopcock (~3 feet).
6. Place the open end of the Tygon tubing into a flask containing the acrylamide solution, and place the flask about 2 feet above the level of the multi-caster.
7. Close the stopcock to the multi-casting chamber. Fill the tubing to the syringe by drawing up about 20 ml monomer solution with the syringe.
8. Close the stopcock to the acrylamide flask, then open the stopcock to the chamber. Fill the tubing to the chamber with acrylamide solution.
9. Close the stopcock to the syringe and let the multi-casting chamber fill by gravity feed. Adjust the height of the flask if necessary to speed up or slow down the rate of flow. Fill the chamber to a height approximately 1.0 cm below the top of the inner (shorter) glass plates.
10. Close the stopcock to the chamber. Clamp off the tubing from the multi-casting chamber port.
11. Place the syringes filled with overlay solution upright into the gel sandwiches. ***Slowly*** overlay all the gels at the same time by pressing on all of the syringe plungers at once with a flat plate. Remove the syringes, and cover the chamber with a plate to prevent dust from settling on the gels. Let any excess acrylamide flow back into the flask and rinse out the Tygon tubing.

12. Allow polymerization to occur ***undisturbed*** for at least 2 hours.
13. The second dimension slab gels can be stored at 4 °C for up to 1 week. To store the gels, remove them from the casting chamber and pour off the overlay solution. Clean any excess acrylamide from the glass plates, and rinse the tops of the gels with ddH_2O. Wrap the gels tightly in Saran Wrap and store them in an airtight plastic container to prevent drying.

3.4 Assembly of Resolving Gels

1. Remove the previously cast resolving gels from storage and fit them with clamps so that the arrows on the clamps face inward and the tops of the plates meet the corresponding notches in the clamps as shown in Figure 3.1. Tighten clamp screws.

Fig. 3.1.

Note: *This protocol does not use a stacking gel.* However, if a stacking gel is required for a particular application, it should be cast on a level surface. It is important that the same amount of monomer be used for each stacking gel to insure stacking gels of identical depth. If a comb is not used, as in most 2-D applications, then the stacking gel should be overlaid with 1.0 ml of water saturated sec-butanol. After polymerization is complete, drain off the overlay (or remove the combs), and rinse the gel surface briefly with distilled deionized water.

3.5 Preparing PROTEAN II xi multi-cell for Second Dimension Run (requires 2-D conversion kit)

1. Place the central cooling cores into the tank so that the cathode posts are all correctly aligned along one side. Place the two cooling coils between the cooling cores so that they line up with the notches in the cell lid. Using the cooling manifold, connect both the cooling cores and cooling coils to a refrigerated water recirculator.

2. Make sure the spigot on the bottom of the tank is firmly attached to the tank, and that it is in the off position. Pour the second dimension running electrolyte into the lower buffer tank. For electrical safety, the lower buffer level must always be at least 3 cm below the top of the inner glass plates. We recommend that you add sodium azide to the lower tank buffer. With sodium azide in the lower buffer tank the buffer can be retained and reused for up to 3 months depending upon the frequency of use and whether or not the cooling system is allowed to run continuously. This reduces the time and expense of repeatedly producing large quantities of buffer. CAUTION: Be sure to wear gloves when handling lower electrolyte buffer as sodium azide is poisonous.

   For optimal results, the water chiller should be started at least 2 hours prior to operating the PROTEIN II xi multi-cell to insure that the system is between 8-12 °C.

3.6 Attaching the Gel Sandwiches

Important: Before turning off the power to the focusing cell apparatus, the transfer solution and all necessary buffers should be prepared and the second dimension resolving gels should be set up attached to the central cooling cores, and ready for the tube gels. The tube gel must be extruded and applied to the resolving gel as quickly as possible to avoid diffusion of focused proteins.

Note: To insure a good upper buffer seal, the entire gasket on the central cooling core should be lubricated with water or upper electrolyte prior to attaching the gel sandwich.

1. With the short glass plate facing the cooling core (and the locating decal on the clamps facing the core) position the gel sandwich so that the grooves in the upper portion of the clamps line up with the locating pins on the central cooling core. The gel sandwich will be at approximately a 30° angle, with the outer glass plate against the U-shaped gasket and the tabs of each clamp against the white latch assembly on the side of the cooling core (Figure 3.2).

   Note: For purposes of illustration, the cooling manifold has been removed.

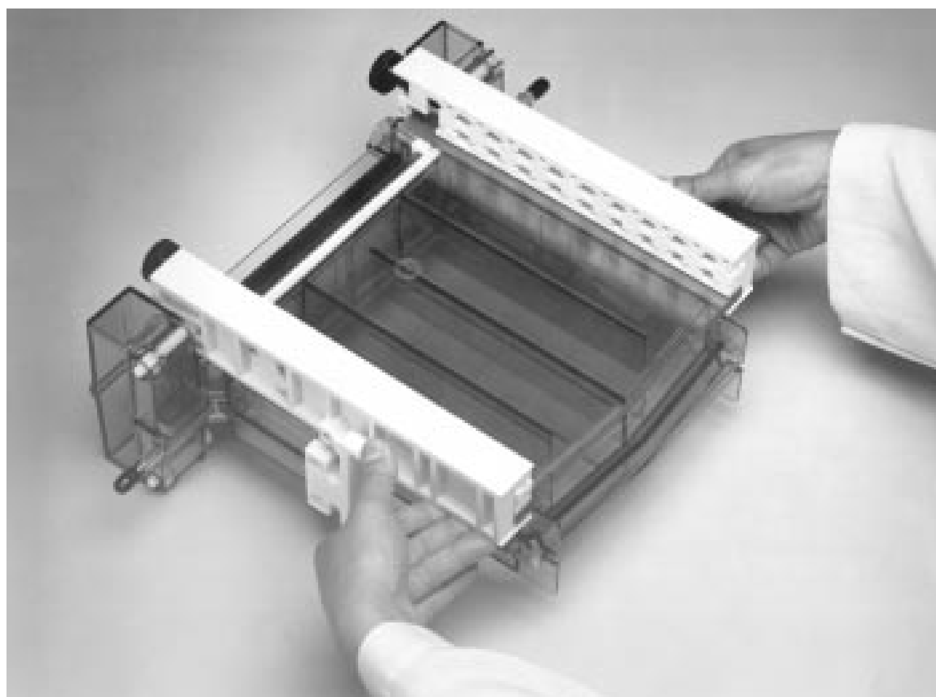

Fig. 3.2.

2. With your fingers on the inside of the latch on the cooling core and your thumbs resting on the clamps, gently snap the gel sandwich onto the cooling core (Figure 3.3).

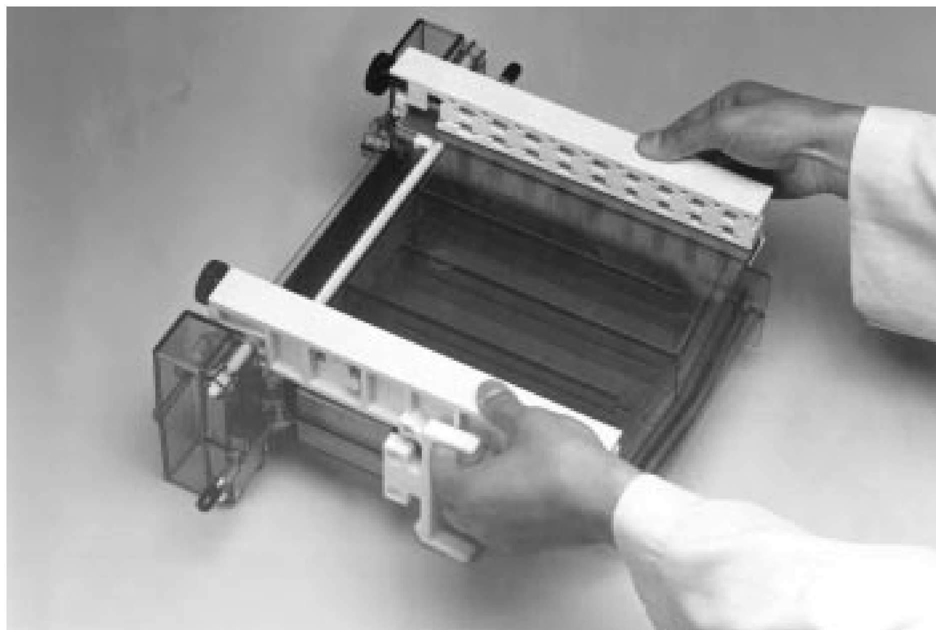

Fig. 3.3.

3. Repeat steps 1 and 2 to attach the second gel sandwich to the other side of the cooling core.

   Repeat these steps with the remaining gels and cooling cores.

   Note: If only one gel is to be run on one of the cores, the supplied upper buffer dam must be attached to the cooling core on the other side to form the complete upper buffer chamber. Position the acrylic plate between two clamps by sliding the dam up into each clamp as far as possible. No further alignment is necessary. The acrylic buffer dam fits both the 16 and 20 cm clamps.

3.7 Tube Gel Extrusion and Application

Note: In this protocol, no equilibration of tube gels is required prior to the second dimension run.

1. Prepare a few syringes for ejecting the tube gels by attaching small pipet tips to the ends of 1 ml syringes. It may be necessary to cut the top of the pipet tip for a snug fit onto the syringe. Fill the syringes with deionized H_2O. Prepare a transfer sheet by cutting a piece of Parafilm (or one of the extra plastic separation sheets included with the multi-casting chamber) to a size that will fit between the spacers of the gel sandwich. This will be used to support extruded tube gels, while transferring them to the second dimension gel. Also, have ready a pipettor set at 150 µl and a clean spatula.
2. Remove all water from the tops of the resolving gels with a piece of filter paper.
3. Turn off the power supply and drain the upper buffer from the tube gel apparatus.

4. Remove a tube gel from the apparatus. Rinse the NaOH off the top of the tube gel before extrusion with syringe and needle. Using the 1 ml H_2O filled syringe, fit the pipet tip into the hole on the basic side of the capillary tube. Press on the plunger and extrude the gels onto the Parafilm or plastic sheet. Gently blot around the gels with an absorbent tissue and remove all excess water from the transfer sheet.
5. Pipet 150 µl of transfer solution (described in Section 7) along the length of the extruded gel.
6. Using a spatula, immediately slide the capillary gel in between the glass plates onto the second dimension gel working from one end to the other (see Figure 3.4). Care must be taken to avoid entrapment of air bubbles between the two gels. Make sure that the entire length of the gel is in contact with the resolving gel.
7. Repeat this procedure for the remaining gels. No agarose solution is necessary for sealing the IEF gels.

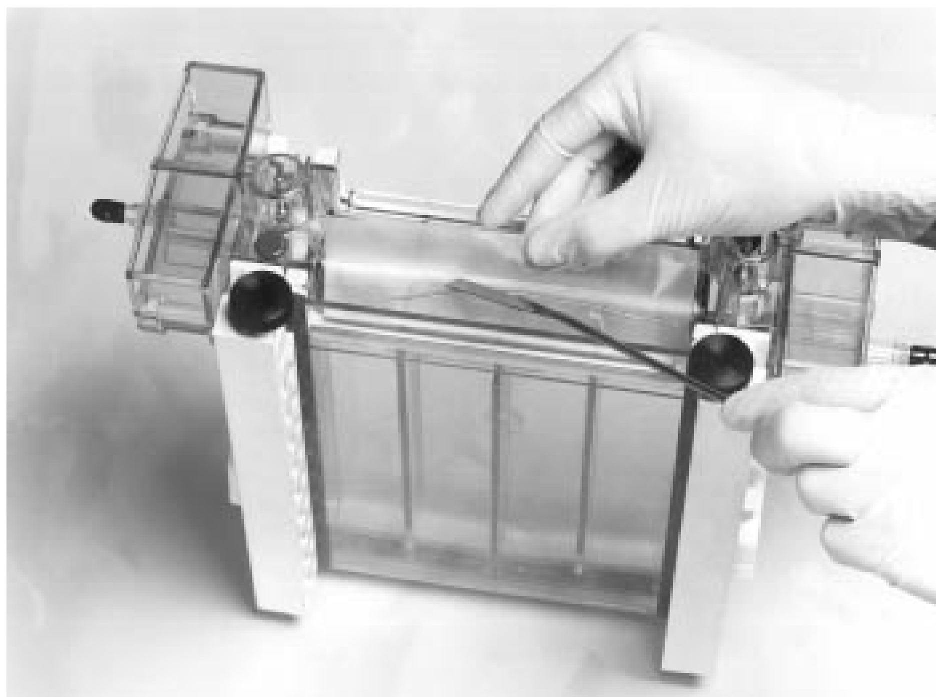

Fig. 3.4.

3.8 Running the Second Dimension

1. Place the three central cooling cores into the lower buffer chamber.
2. Put 350 ml of running electrolyte (Section 7) into each of the upper buffer chambers. (Azide is not required. Do not reuse this buffer.)
3. Place the lid on top of the lower buffer chamber to fully enclose the PROTEAN II xi 2-D multi-cell. Note that the lid can be placed in only one orientation, so that the anode and cathode connections cannot be reversed.
4. Attach the power cables to a suitable power supply, making sure that the polarity is correct.
5. Run the gels at a constant current of 40 mA per slab gel; Bio-Rad's Model 1000/500 Power Supply is recommended. At the end of the run, remove the gels from the glass plates for staining.

Section 4
Silver Staining Procedure

We recommend Bio-Rad's Silver Stain Plus Kit, catalog number 161-0449.

Silver Stain Plus is an adaptation of the Gottlieb and Chavko[6] method used to stain proteins and nucleic acids in polyacrylamide and agarose gels. This is an ultrasensitive protein stain for 2-D gels, IEF gels, and vertical slab gels. Silver Stain Plus is an ideal alternative to ethidium bromide staining of nucleic acids in submarine gels and CHEF gels.

The Silver Stain Plus Kit offers the advantages of a 1 hour, 3 step procedure, premixed solutions, and nanogram detection of proteins and nucleic acids.

Section 5
Maintenance of Equipment

PROTEAN II xi multi-cell chamber, cores and clamps	Rinse thoroughly with distilled water after every use.
Glass plates, spacers, combs	Wash with a non-abrasive laboratory detergent then rinse thoroughly with distilled water. Glass plates can be rinsed with ethanol to prevent water spots from forming.
Glass plates (if more stringent cleaning is required)	Soak in a strong acid solution (chromic acid/sulfuric acid cleaning solution) for ≥30 minutes and then rinse thoroughly with distilled water.*

Warning: Exercise extreme caution for acid cleaning; wear safety glasses, a lab coat, and rubber gloves. Keep a container of $NaCO_3$ nearby to neutralize spills.

Glass tubes	After use, rinse with laboratory detergent solution, scrub out if possible, then rinse with distilled H_2O. Alternatively, soak in chromic/sulfuric acid solution. Rinse thoroughly with distilled water and dry in forced air vacuum oven before use. When the chromic/sulfuric acid solution turns green, it is used up and should be properly discarded with chemical waste.

*An alternative to chromic/sulfuric acid cleaning is methanolic KOH (5 g KOH in 100 ml methanol). It is very caustic and will break down protein and polyacrylamide on the plates or in the tubes.

Section 6
Troubleshooting Guide - 2-D PAGE

Problem	Cause	Solution
1. "Smile" effect spot pattern curves upward at both sides of the gel.	a. Center of the gel running hotter than either side.	a. Make sure coolant is circulating at 8-12 °C.
	b. Power conditions excessive.	b. Decrease power setting.
2. Diffuse tracking dye.	a. Decomposition of transfer solution and/or buffer stock solutions.	a. Prepare fresh reagents. Shelf life of aqueous solutions is 30 days at 4 °C for buffer and monomer stocks.
	b. Diffusion.	b. If protein spots and tracking dye bands are diffuse, increase current by 25-30% and/or increase %T of resolving gel.*
3. Vertical streaking of spots.	a. Sample overload.	a. Dilute sample, selectively remove predominant protein in the sample, or reduce current about 25% to minimize streaking.
4. Horizontal streaking of spots.	a. Sample overload.	a. If urea/nonionic detergent is not sufficient, use SDS. Centrifugation of sample may be necessary (up to 100,000 x g for 30 minutes) to remove undissolved particulates.
	b. Interfering nucleic acids.	b. Treat sample with DNase or RNase.
	c. Focusing is incomplete.	c. Increase power to 1000V for 2 hours.
5. Artifactual Heterogeneity.	a. Urea may not solubilize some proteins; can cause carbamylation and decrease in pIs or artifactual molecular weights.	a. Use pure urea freshly prepared or frozen as aliquots. Avoid excess heat.

*Polyacrylamide gels are described by reference to two properties:
1) The total monomer concentration, (%T)
2) The crosslinking monomer concentration (%C)

$$\%T = \frac{\text{g acrylamide + g bis-acrylamide}}{\text{total volume}} \times 100$$

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

Problem	Cause	Solution
6. Broad or diffuse spots.	a. Diffusion due to slow migration in 2nd dimension.	a. Increase current by 20%.
	b. Chemical changes due to ionic contaminants in urea.	b. Deionize urea.
7. Skewed or distorted bands.	a. Poor polymerization in gel.	a. Degas gel solution thoroughly prior to casting; increase ammonium persulfate and TEMED concentrations by 25%, or add riboflavin phosphate to 5 µg/ml in addition to the usual catalyst levels in the gel.
	b. Salts in sample.	b. Remove salts by dialysis, desalting column, etc.
	c. Uneven gel interface.	c. Increase reaction rate, overlay carefully.
	d. Overheating due to inadequate coolant flow rate.	d. Increase flow rate or reduce current by 25-30%.
8. Run taking unusually long time.	a. Buffers too concentrated.	a. Check buffer protocol, dilute if necessary.
	b. Low current.	b. Increase current by 25-50%.
9. Run too fast, poor resolution.	a. Buffer too dilute.	a. Check buffer protocol, concentrate if necessary.
	b. Current too high.	b. Decrease current by 25-50%.

Section 7
Gel Protocols

7.1 Stock Solutions

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

Dissolve 146 g acrylamide and 4 g N′N′-bis-methylene-acrylamide in 350 ml deionized water. Make to 500 ml with distilled water. Filter through 0.45 µm pore size filter and store at 4 °C in the dark (30 days maximum).

Or substitute Bio-Rad's Preweighed Acrylamide/Bis

37.5:1 mixture (catalog number 161-0112, 30 g)
(catalog number 161-0106, 200 g)

Dissolve 150 g acrylamide/bis (30 g/100 ml) in 500 ml deionized H_2O.

B. 1.5 M Tris-HCl, pH 8.8

Dissolve 54.45 g Tris base in ~150 ml deionized water.

Adjust to pH 8.8 with HCl. Make to 300 ml with deionized water and store at 4 °C.

C. 10% SDS

Dissolve 10 g SDS in ~60 ml of water with gentle stirring and bring to 100 ml with dH_2O.

7.2 First Dimension IEF Tube Gels

Detergent Solution

0.3 g CHAPS
100 µl Nonidet P-40
900 µl ddH_2O
Dissolve CHAPS in water then add Nonidet P-40.

First Dimension Capillary Tube Gel Monomer Solution

11 g urea (9.2 M final concentration)
3 ml acrylamide/bis stock (4.5% total monomer)

Stir to dissolve the urea. This step is done slowly without heating or with very gentle heating only.

0.2 ml Bio-Lyte® 5/7 ampholyte
0.8 ml Bio-Lyte 3-10 ampholyte
1 ml detergent solution (CHAPS/NP-40) from above
6 ml D1 H_2O

Add deionized water to 20 ml

Mix and degas.

Add 20 µl TEMED after degassing to avoid evaporation.

Add 40 µl 10% (w/v) APS, swirl 8-10 times and cast the gels.

This makes 20 ml total volume, enough to cast one set of gels.

Sample Solution A

1.0 g SDS
0.232 g DTT or DTE

Dissolve in ddH_2O to a final volume of 10 ml. Store in aliquots at -70 °C.

Iso-Urea Solution E

0.1 g DTT
0.4 g CHAPS
5.4 g urea
500 µl Bio-Lyte 3/10 ampholyte
50 µl 0.5% bromophenol blue
6 ml ddH_2O

7.3 Electrolytes

First Dimension Upper Running Electrolyte - Cathode (20 mM NaOH)

Dissolve 0.4 g NaOH in 500 ml deionized water and degas thoroughly for 30 minutes.

First Dimension Lower Running Electrolyte - Anode (10 mM H_3PO_4)

Dilute 1.8 ml concentrated H_3PO_4 in 2.6 liters deionized water and degas thoroughly for 30 minutes.

7.4 Transfer Solution

40 ml 0.5 M Tris-HCl, pH 8.8
80 ml 10% SDS
8 ml 0.05% bromophenol blue
150 ml ddH_2O

7.5 Second Dimension Resolving Gels

Second Dimension Gel Solution

(To cast more than nine 1.5 mm gels, increase volume proportionately.)

214 ml ddH_2O
190 ml 1.5 M Tris-HCl, pH 8.8
310 ml acrylamide/bis stock solution

Mix and degas.

Add 175 µl TEMED after degassing to avoid evaporation.

Add 1.75 ml 10% APS and gently swirl 8-10 times.

Note: In the Hochstrasser et al. procedure, SDS is left out of the resolving gel.

Second Dimension Running Electrolyte

20 g SDS
120 g Tris
576 g Glycine

Add ddH_2O to a final volume of 20 liters.

For the lower buffer, add 4 g sodium azide/20 liters. With the cooling system operating continuously, the lower buffer can be kept in the multi-cell lower chamber for up to 3 months depending upon the frequency of use. For upper buffer, do not include azide and do not reuse.

Section 8
Two-Dimensional Gel System References

1. Hochstrasser, D. F., Harrington, M. G., Hochstrasser, A. C., Miller, M. J. and Merril, C.R., *Analytical Biochemistry,* **173,** 424-435 (1988).
2. Hochstrasser, D. F., Augsburger, V., Funk, M., Appel, R., Pelegrini, C. and Muller, A. F., *Electrophoresis,* **7,** 505-511 (1986).
3. Oakley, et al., *Analytical Biochemistry,* **105,** 361-363 (1980).
4. Wiederkehr, et al., *Clinical Chemistry,* **31,** 1537-1542 (1985).
5. Merril, C. R., Goldman, D. and Van Keuren, M. L., *Methods in Enzymology,* **96,** 230-239 (1983).
6. Gottlieb, M. and Chavko, M., *Analytical Biochemistry,* **165,** 33-37 (1987).

Section 9
Equipment and Accessories

Product Description	Catalog Number

9.1 PROTEAN II xi Multi-Cell Configurations

PROTEAN II xi Multi-Cell, includes 3 central cooling cores with gaskets, lid with power cables, tank, 1 upper buffer dam, PROTEAN II xi multi-gel casting chamber with accessories, leveling bubble, and instructions	165-1951

9.2 Additional Required Equipment (not included)

-Refrigerated water recirculator, 100/120 VAC or 220/250 VAC	
-Capillary tubes (1.5 mm ID, 7.5 mm OD, 180 mm)	
-1 ml syringes (tuberculin type works well)	
-Pipet tips	
-Water saturated sec-butanol	
-Tygon tubing pieces, 12 (3/16" ID x 1/4" OD, 2 cm length)	
-Disposable test tubes, 12 (12 x 75 mm)	
-Filter paper	162-0118
-Stopcock, 3-way	

9.3 Accessories

PROTEAN II xi Multi-Cell 2-D Conversion Kit, includes cooling coils, manifold to convert 165-1951 into high resolution 2-D configuration	165-1956
PROTEAN II xi Multi-Cell Lid	165-1959

Glass Plates

Inner Plate, 2	165-1821	165-1823
Outer Plate, 2	165-1822	165-1824
Frosted Inner Plate, 2 (agarose gels)†	165-1825	165-1826
Beveled Inner Plate, 2 (2-D procedures)†	165-1827	165-1828

† Used in conjunction with regular outer plate.

Plate Washer System

PROTEAN II xi Plate Washer System	165-1991
PROTEAN II xi Plate Holder	165-1992
Bio-Rad Cleaning Concentrate	161-0722

Spacers (set of 4)

0.5 mm	165-1841	165-1846
0.75 mm	165-1842	165-1847
1.0 mm	165-1843	165-1848
1.5 mm	165-1844	165-1849
3.0 mm	165-1845	165-1850

Product Description	Catalog Number
DH: 9 Combs (each)	
25 well x 0.75 mm, well volume 60 µl	165-1861
25 well x 1.0 mm, well volume 80 µl	165-1862
25 well x 1.5 mm, well volume 120 µl	165-1863
20 well x 0.5 mm, well volume 54 µ	165-1865
20 well x 0.75 mm, well volume 82 µ	165-1866
20 well x 1.0 mm, well volume 110 µ	165-1867
20 well x 1.5 mm, well volume 164 µl	165 1868
20 well x 3.0 mm, well volume 328 µl	165-1869
15 well x 0.5 mm, well volume 74 µl	165-1870
15 well x 0.75 mm, well volume 110 µl	165-1871
15 well x 1.0 mm, well volume 147 µl	165-1872
15 well x 1.5 mm, well volume 221 µl	165-1873
15 well x 3.0 mm, well volume 442 µl	165-1874
PTFE Combs (cont.)	
10 well x 0.5 mm, well volume 114 µl	165-1875
10 well x 0.75 mm, well volume 172 µl	165-1876
10 well x 1.0 mm, well volume 229 µl	165-1877
10 well x 1.5 mm, well volume 343 µl	165-1878
10 well x 3.0 mm, well volume 687 µl	165-1879
5 well x 1.0 mm, well volume 522 µl	165-1882
5 well x 1.5 mm, well volume 783 µl	165-1883
5 well x 3.0 mm, well volume 1.57 ml	165-1884
3 well x 1.5 mm, well volume 1.37 ml	165-1888
Blank x 0.75 mm, well volume 2.44 ml	165-1891
Blank x 1.0 mm, well volume 3.26 ml	165-1892
Blank x 1.5 mm, well volume 4.88 ml	165-1893
Blank x 3.0 mm, well volume 9.76 ml	165-1894
2-D x 1.0 mm, 1 ref. well, well volume 79 µl (ref. well)	165-1897
2-D x 1.5 mm, 1 ref. well, well volume 119 µl (ref. well)	165-1898
2-D x 3.0 mm, 1 ref. well, well volume 238 µl (ref. well)	165-1899
Sandwich Clamps, Dams, Gaskets, Inserts	
Clamps, 16 cm set, 1 (one left, one right)	165-1901
Clamps, 20 cm set, 1 (one left, one right)	165-1902
Upper Buffer Dam, 1	165-1909
Replacement Gaskets, central cooling core, 2	165-1913

9.4 Multi-Gel Casting Chamber and Accessories

PROTEAN II xi Multi-Gel Casting Chamber	165-2025
Sealing Gasket	165-2026
Acrylic Blocks, 4	165-1957
Separation Sheets, 15	165-1958

Product Description	Catalog Number

9.5 Electrophoresis Chemicals

Coomassie Blue R-250, 10 g	161-0400
Bromophenol Blue, 10 g	161-0404
Triton X-100, 500 ml	161-0407
CHAPS, 1 g	161-0460
CHAPSO, 1 g	161-0465
Urea, 250 g	161-0730
Urea, 1 kg	161-0731
AG® 501-X8 Ion Exchange Resin, 1 lb	142-6424

Bio-Lyte® Ampholytes

Bio-Lyte 3/10 Ampholyte, 40%, 10 ml	163-1112
Bio-Lyte 3/5 Ampholyte, 20%, 10 ml	163-1132
Bio-Lyte 4/6 Ampholyte, 40%, 10 ml	163-1142
Bio-Lyte 5/7 Ampholyte, 40%, 10 ml	163-1152
Bio-Lyte 5/8 Ampholyte, 40%, 10 ml	163-1192
Bio-Lyte 6/8 Ampholyte, 40%, 10 ml	163-1162
Bio-Lyte 7/9 Ampholyte, 40%, 10 ml	163-1172
Bio-Lyte 8/10 Ampholyte, 20%, 10 ml	163-1182

Large Volume Ampholytes

Bio-Lyte 3/10 Ampholyte, 40%, 25 ml	163-1113
Bio-Lyte 4/6 Ampholyte, 40%, 25 ml	163-1143
Bio-Lyte, 5/7 Ampholyte, 40%, 25 ml	163-1153
Bio-Lyte, 5/8 Ampholyte, 40%, 25 ml	163-1193
Bio-Lyte 6/8 Ampholyte, 40%, 25 ml	163-1163

9.6 Power Supplies

PowerPac 3000 Power Supply, 100/120 VAC	165-5056
PowerPac 3000 Power Supply, 220/240 VAC	165-5057
PowerPac 1000 Power Supply, 100/120 VAC	165-5054
PowerPac 1000 Power Supply, 220/240 VAC	165-5055

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