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USING THE SCIE-PLAS VERTICAL GEL ELECTROPHORESIS UNITS

A. Safety Precautions

- READ the instructions before using the apparatus.
- Always isolate electrophoresis units from their power supply before removing the safety cover. Isolate the power supply from the mains FIRST then disconnect the leads.
- DO NOT exceed the maximum operating voltage or current (see table 1).
- DO NOT operate the electrophoresis units in metal trays.
  Acrylamide is a volatile, cumulative neurotoxin and suspected carcinogen. Wear effective protective clothing and follow recommended handling and disposal procedures.
- Polymerised gels contain some unpolymerised monomer. Handle with gloves only.
  Following the replacement of a platinum electrode have the unit inspected and approved by your safety officer prior to use.
- DO NOT fill the unit with running buffer above the maximum fill lines.
- DO NOT move the unit when it is running.
- CAUTION: During electrophoresis very low quantities of various gases are produced at the electrodes. The type of gas produced depends on the composition of the buffer employed. To disperse these gases make sure that the apparatus is run in a well-ventilated area.

B. General Care and Maintenance

- To remove the safety lid, push thumbs down on the plastic lugs and lift the lid vertically with your fingers.
- Before use clean and dry the apparatus with DISTILLED WATER ONLY. IMPORTANT: Acrylic plastic is NOT resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohol's (over 25%) and acids (over 25%), they will cause "crazing" especially of the UV transparent plastic and should NOT be used for cleaning. DO NOT use abrasive creams or scourers. Dry components with clean tissues prior to use.
- Before use, and then on a monthly basis, check the unit for any leaks at the bonded joints. Place the unit on a sheet of dry tissue and then fill with DISTilIED WATER ONLY to the maximum fill line. Any leakage will be seen on the tissue paper. If any leakage is seen DO NOT ATTEMPT TO REPAIR OR USE THE APPARATUS, but notify Topac immediately.
- The replacement platinum electrodes are partially shrouded for protection. However, when cleaning the main tank DO NOT use cleaning brushes in the electrode area. Usually a thorough rinse with distilled water is all that is required.
- Ensure that the connectors are clean and dry before usage or storage.

C. Storage of Water Cooled Units Note not on TV100. See TV100K and TV100YK Water cooled units can be stored with water in the base core but 0.02% sodium azide should be added to prevent algal gj'owth. Store in a dark cupboard or cold room. Alternatively, drain the unit. A small quantity of water will remain in the base core. If algal growth does build up over a period of time fill the base core with neutral Decon overnight and then flush through with clean water.

D. Filling the Base Cooling Core: Note not on TV100.

The base cooling core will already contain a small quantity of water from control tests. The base cooling core can be used **in** two ways. Static water can be used as a simple heat sink or the tank can be actively regulated using flowing water from a tap or water bath.

Static Temperature Regulation:

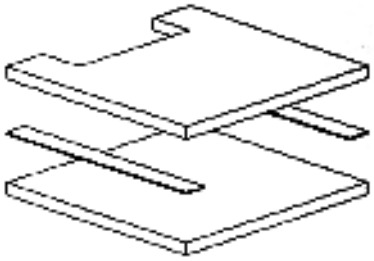
1. Attach a short length of rubber hose to each connector.
2. Incline the unit at an angle of approximately 45 degrees with the ports uppermost
3. Use a funnel to fill the cooling core with de-ionized water containing O.O2%(w/v) sodium azide (preservative to prevent bacterial and algal growth)
4. When filled, keep the unit inclined and attach clamps to each piece of rubber hose. 5. The unit can be cooled before an electrophoresis run if required. **DO NOT FREEZE.**

Active Temperature Regulation:

1. Attach a short length of rubber hose to each connector.
2. Attach one end of the rubber hose to the outlet port of a circulating water bath and the other end to the inlet port. Alternatively attach one end of a rubber hose to a water supply and allow the other rubber hose drain to waste.
3. **The maximum recommended water flow rate is 1 Litre/min. DO NOT exceed this figure.**
4. If you are using a circulating water bath, which exceeds this flow rate, you can attach a T-connector in line. One branch of the connector can return water to the bath and the other can flow to the cooling core and incorporate a flow regulator such as an adjustable tubing clamp. Measure and adjust the flow rate before attaching the line to the gel unit

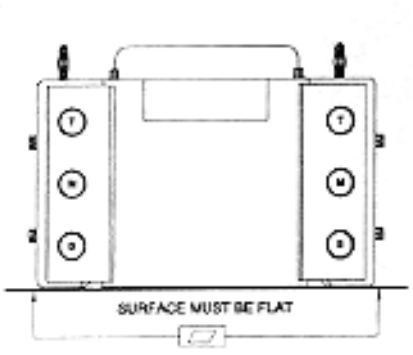
E. Gel Plate Preparation

1. Clean the plates, spacers and combs in mild laboratory detergent **DO NOT** use abrasive creams or scourers. If a particularly clean finish is required (e.g. for silver-stained gels) glass plates can be soaked in chromic acid overnight, rinse with water then wipe successively with ethanol, acetone and ethanol again. **NEVER** allow organic solvents or chromic acid come into contact with the plastic components
2. The notched glass plate can be siliconised in a fume hood with Dimethyldichlorosilane if required to assist in plate separation after the run.
3. Handle clean plates with gloved hands (remove any fingerprints with acetone).



F. Gel Plate Assembly

1. On a clean level bench position the two side spacers flush with the edges of the large glass plate and overlay the small or notched plate, if used.
   See Figure 1.
2. The gel plates can be sealed either by using a gel casting unit, with tape, or by clamping greased spacers between the plates with bulldog clips (see below 5. and Figures 3-10 on pages 10 and 11 for instructions on the Scie-Plas Gel Casting Unit).
3. For tape sealing hold or clamp the plates firmly and seal the edges of the gel cassette with gel sealing tape. The tape should be applied smoothly with no wrinkles. Reinforce the corners by overlapping extra pieces of tape onto the glass. Grease or fingerprints will prevent a good seal being formed.



4. If greasing is the method of choice, smear a little silicone grease or Vaseline over the spacers prior to assembly, use the long spacer to seal the bottom of the gel and clamp with bulldog clips. Note that the side spacers will be too long if this sealing method is chosen. The side spacers should be cut to size - make sure the cut is a clean right angle.
5. When using the Scie-Plas Gel Casting Unit, first make sure that the bottom edges of the glass

plates are ground and free from any cracks or chips. Loosen the gel plate retaining screws of the Central Gel Running Module and pull out the clamping bars until they are in a fully open position.

6. Insert the gel plate assemblies into the Central Gel Running Module, notched plate inner most, and place on a flat surface, as shown in Figure 2. The Gel Casting Base turned upside down makes an ideal flat surface (see figure 3, page 10). Note: Both plate assemblies must be inserted, then tightened simultaneously. When only casting or running one gel, the dummy plate provided must be fixed on the other side otherwise the casting unit will bow when the screws are tightened causing leakage. Make sure that the bottom edges of the spacers are in line with the bottom edges of the glass plates. Use the spacer aligner to keep the spacers straight and upright. Loosely tighten the screws FOR BOTH GEL PLATE ASSEMBLIES SIMULTANEOUSLY in the following order, Middle (M), Bottom (B), Top (T) as shown in figure 2. Top then bottom for TV100. Press on the tops of the plates and keep tightening the screws in this order until they are all finger tight. Check again that the bottom edges of the spacers and glass plates are perfectly aligned. If they are not then loosen the screws and realign.
7. Place the Central Gel Running Module on the Casting Base, with the silicone mats fully covering the bottom edges of the glass plates, and insert the cam pins with the handles facing downwards (fig 4).
8. Turn the cams until appreciable pressure can be felt and never more than -1700. DO NOT OVER TIGHTEN passed this point as this will only force the glass plates up off the silicone seals.

G. Gel Pouring

1. For reproducibility and uniform polyacrylamide cross-linking we recommend deionising, degassing and filtration of acrylamide gel solutions prior to use. Acrylamide solutions should be stored in a cool, dark environment such as a refrigerator and allowed to reach room temperature prior to pouring. Avoid exposure to heat and sunlight
2. Polymerization conditions should be adjusted to effect polymerization within about 5 - 10 minutes. Test a small volume in a vial prior to pouring the gel. As a rough guide 100ml of degassed 6% acrylamide gel will set in about 5 minutes at room temperature when gently mixed with 450ul of freshly prepared 10% (w/v) Ammonium persulphate plus 200ul TEMED. The setting time increases to about 10 minutes if the TEMED volume is reduced to 100ul and to approximately 15 minutes with 75ul. The amount of catalysts may need to be reduced under warm conditions. Do not pour under direct sunlight Gel pouring can be carried out directly in a Scie-Plas Gel Casting Unit or by clamping a taped gel into the tank unit
3. Run the acrylamide separating gel mix slowly down the inside edge of the gel cassette. Avoid aeration. If a stacking gel is to be used, carefully overlay the separating get to a depth of 3 - 5mm with 1 x separating gel buffer or water-saturated butanol.
4. Following polymerization of the separating gel, pour off the overlay layer (rinse off butanol with electrophoresis gel buffer) and pour a stacking gel if required.
5. Insert the comb ensuring bubbles are not trapped. Once the stacking gel has polymerised use the gel immediately or store wrapped in a damp paper towel and Clingfilm at 4°C.
6. Remove any tape (if used) from the bottom of the gel and from any region that could affect the seal between the glass and the silicone gasket Clean both the silicone gasket, located on the upper buffer chamber, and the outside of the gel plates If the gasket becomes unseated from its groove simply press it back into place.
7. Clamp the gel plates into position in the unit with the short or notched plate innermost, using the clamps provided. DO NOT OVER TIGHTEN as this will cause the glass plates to crack.
8. When using the Central Gel Running Module for casting, remove the cams to release the running module and gels. Do not release the clamping screws. Wash off any residual acrylamide. Place the inner running module into the running tank
9. Carefully remove the comb ensuring not to damage the wells.
10. Add the appropriate volume of running buffer to the upper and lower chambers (see table 1), replace the lid and connect to a suitable power supply
    IMPORTANT: DO NOT fill over the Maximum Fill Lines. NOTE: When running only one gel, in any of the dual systems, a dummy plate is required on the other side of the unit, to retain the top buffer level.

Pre-Cast Gels

1. Insert into the Central Gel Running Module notched plate on the inside and tighten the screws as advised above.

H. Gel and Buffer volumes/run conditions

Some guidelines for operating conditions are given in Table 1 but conditions vary according to the number of gels, their composition, length and cross sectional area. The current required will increase in proportion to the number of gels or gel thickness providing that the voltage is not limiting, e.g. 2 gels require twice the current of 1 but the same voltage. Longer gels require proportionally higher voltages. By increasing the gel concentration the electrical resistance is increased and the rate of migration decreases Higher voltages can be applied but be careful not to overheat the gel. The conductivity (non-dissociating buffer systems gels vary enormously and conditions have to be determined empirically.

Table 1.

Operating conditions for 10cm and 20cm long vertical gels with 1mm spacers

Model	Buffer Volume (ml)	Approx gel Volume (ml)	Voltage (Volts)	Current (mAmps)
V10-SET	Upper 60 Lower 65	7	125-180	15-30
V10-CDC	Upper 120	7	150-225	25-45 (1 gel)
	Lower 1120			50-85 (2 gels)
TV100	Upper 100	7	150-225	25-45 (1 gel)
	Lower 1000			50-85 (2 gels)
V10-WCDC	Upper 270	17	150-225	55-85 (1gel)
	Lower 2000			110-160 (2 gels)
V20-SET	Upper 325	35	90-120	(Stacking Gel)
	Lower 225		120-180	(Resolving Gel) 20-30 (Per Gel)
V20-CDC	Upper 650	35	90-120	(Stacking Gel)
	Lower 3500		120-180	(Resolving Gel) 20-30 (Per Gel)

2. The run conditions are to be taken as a guideline only and apply to SOS Tris-glycine gels.
   If the plates become hot increase the water flow rates within the recommended limits or reduce the power settings.
3. If a native gel is being used, pre-electrophorese the gel for 15 - 40 minutes prior to loading.
4. For SOS gels do not pre-electrophorese the gel.

I. Sample Loading

1. Centrifuge samples at 12,000 x g for 5 minutes. If this stage is omitted samples may streak during electrophoresis.
2. Carefully remove the sample comb and immediately flush the wells with electrophoresis buffer from a syringe.
3. Load the samples using a gel loading pipette tip. If possible avoid taking liquid from the pellet area at the bottom of the tube. During sample loading the pipette tip should be 1 - 2mm above the bottom of the well to minimize dilution of the sample and to keep the sample as a tight layer.
4. Fill unused wells with the equivalent volume of sample buffer to maintain uniform electrical resistance across the gel.
5. Replace the safety lid firmly making sure that the electrical connectors form a good contact.
6. Connect the electrophoresis apparatus to the power pack and connect the power pack to the mains supply. Turn all settings to zero before turning on the mains supply. Adjust the controls to the desired settings. Follow manufacturer's instructions.

Table 2 provides a guide to the amounts of protein that can be successfully applied to a 5mm wide, 1 mm gel slot.

Table 2.

Comb	Single Band	Multiple Bands	Sample Volume
1 mm x 5mm wide	1 - 6 mg	30 - 60 mg	< 40 ul
1.5mm x 5mm wide	1 - 10 mg	50 - 100 mg	< 60 ul

The volumes of sample wells can be estimated by simply multiplying the comb tooth width and thickness by the depth of the well.

J. At The End of the Run

Turn the power supply settings to zero, turn off the mains supply and disconnect the power leads. Turn off the water supply if the unit is being cooled.

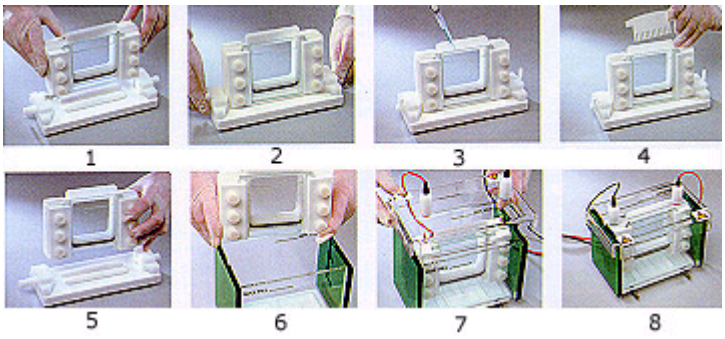
Remove the safety lid by gripping the handles and pressing on the locating lugs with your thumbs.

After unclamping the gel and removing the tape (if used), separate the plates with a strong broad blade. If you are using notched or eared plates DO NOT prise them apart at the ears. Spread the load over a wide area.

After removing the gel for staining or blotting, clean the plates thoroughly and rinse in distilled water. A clean sheet of foam rubber placed at the bottom of the sink serves as a usual support and minimizes the risk of glass plate damage.

Empty the buffer chambers with a vacuum line and trap or carefully decant the buffer away from the electrical connectors. Rinse the chambers with distilled water then dry the electrode connectors with tissue. Ensure that the connectors are clean and dry before usage or storage.

Casting Gels using the SciePlas Gel Casting Unit



Advance casting of gels not only increases gel throughput but improves reproducibility. The Scie Plas system allows two gels to be cast at a time directly in the Central Gel Running module (either 10 x 10 cm, 20 x 20 cm or 10 x 20 cm) This eliminates the need to disassemble the cast gels prior to placing into a typical gel tank.

Vertical Gel Electrophoresis - Gel Casting method

- 1. With plates in position place central running module in the casting base.
- 2. Turn both cams to pull module down onto silicone sealing units.
- 3. Pour gel between plates.
- 4. Place appropriate comb between plates and allow gels to set.
- 5. After carefully removing combs release module from casting base.
- 6 Place the module in the outer buffer tank and fill the two buffer chambers.
- 7. Load sample and replace lid.
- 8. Unit is now ready for electrophoresis

Troubleshooting Guide

Many factors may affect the quality of vertical gel separations, for example, preparation of gel and sample buffers; gel casting and tank assembly; run conditions. Most problems can be avoided by reading and following the instructions in this operating manual. Below we list some of those most commonly experienced along with suggestions for solving them. If however, these should not resolve the issue, or if you have questions not covered below, please call Topac Technical Support on 781 740 8778

Equipment Problem

	Comments
Acrylamide solution leaks during casting.	
	Ensure that the sealing surfaces of the glass plates and spacers are clean. Ensure that each plate is free of chips. Ensure the spacers are vertical and flush with the lower plate edge. Ensure that each screw on the central running module has been fully tightened.
Acrylamide solution leaks during casting. glass (No casting base).	
	Ensure that the sealing surfaces of the plates and spacers are clean. Ensure that the tape is pressed firmly onto the plates.
Bubbles do not appear on the electrodes	
	Ensure that the DC power supply is operating properly.
Gels fail to polymerise	
	May be caused by low temperatures, oxygen, insufficient / degraded catalyst or low acrylamide concentrations.

Electrophoresis Problem	**Comments**
Vertical streaking	Caused by excessive sample or particles in sample. Either dilute sample or reduce voltage. Centrifuge samples to remove particulate contamination
Bands spread laterally	Caused by diffusion from sample wells prior to run. Reduce time from sample loading to run start.
Distorted sample wells	Incomplete polymerisation produces poorly defined wells. De-gas gel solution prior to casting and increase ammonium persulphate and TEMED concentrations
Curved dye fronts - "smiling"	Caused by gel being hotter in middle than at the edges. Reduce power settings in supply and/or increase gel cooling
Run takes longer than usual	Buffers may be too concentrated or at the wrong pH. Gel

	concentration may be too high. Adjust if necessary. Alternatively increase the voltage.
Poorly resolved bands	May be caused by too much sample for well width or gel thickness - dilute sample. Lower volumes generally give better resolution. Excessively high voltages cause fast run times but poor resolution. Sample may have degraded.
Fewer bands then expected with heavy band at dye front	Caused by more than one band migrating to dye front. Increase total monomer concentration (%T). Sample may have degraded due to incorrect storage and / or contamination.

References

1. Maniatis, 1., E. F. and Sam brook, J. (1982) Molecular cloning A laboratory manual, Cold Spring Harbour laboratory, Cold Spring Harbour, New York.
2. Rickwood, D. and Hames, B.D. (eds.) (1982) Gel Electrophoresis of Nucleic Acids A Practical Approach, IRL Press, Oxford, England.
3. Longo, M.C. and Hartley, J.L. (1986) Focus 83,3.
4. Ausubel, et ai, (eds). (1993) Current Protocols in Molecular Biology. Greene Publishing and Wiley-Interscience, New York.

Comb Specifications:

Part No.	Thickness	No. of Samples	Tooth Width	Tooth Spacing	Sample Volume
V10-C075-10	0.75	10	4	3	30
V10-C075-12	0.75	12	3.75	2.25	28
V10-CO.75-16MC	075	16	2.5	2	18
V10-C1-10	1	10	4	3	40
V10-C1-12	1	12	375	2.25	37
V10-C1-16MC	1	16	2.5	2	25
V10-C1.5-10	1.5	10	4	3	60
V10-C1.5-12	1.5	12	375	2.25	56
V10-C1.5-16MC	1.5	16	2.5	2	37
V10-C2-10	2	10	4	3	80
V10-C2-12	2	12	375	2.25	75
V10-C2-16MC	2	16	2.5	2	50
V20-CO.25-24	0.25	24	475	2	11
V20-CO.25-36MC	0.25	36	2.5	2	6
V20-CO 35-24	0.35	24	475	2	16
V20-CO.35-36MC	0.35	36	2.5	2	8
V20-CO.75-10	0.75	10	11	5.5	82
V20-CO.75-18MC	0.75	18	6	3	45
V20-CO.75-24	0.75	24	475	2	35
V20-CO.75-36MC	0.75	36	2.5	2	18
V20-CO.75-48	075	48	2.35	1	17
V20-C1-10	1	10	11	5.5	110
V20-C1-18MC	1	18	6	3	60
V20-C1-24	1	24	4.75	2	47
V20-C1-36 M C	1	36	2.5	2	25
V20-C1-48	1	48	2.35	1	23
V20-C1.5-10	1.5	10	11	5.5	165
V20-C1.5-18MC	1.5	18	6	3	90
V20-C1.5-24	15	24	475	2	71
V20-C1.5-36MC	1.5	36	2.5	2	37
V20-C1.5-48	1.5	48	2.35	1	35
V20-C2-10	2	10	11	5.5	220
V20-C2-18MC	2	18	6	3	120
V20-C2-24	2	24	4.75	2	95
V20-C2-36MC	2	36	2.5	2	50
V20-C2-48	2	48	235	1	47
SC2-M025-24	0.25	24	0.25	5.6	7
SC2-M025-30MC	025	30	025	4.5	5
SC2-M025-36	0.25	36	0.25	4.0	5
SC2-MO.25-48	0.25	48	0.25	2.8	3
SC2-MO.35-24	0.35	24	0.25	5.6	9
SC2-MO.35-30MC	0.35	30	0.25	4.5	7
SC2-MO 35-36	0.35	36	0.25	4.0	7
SC2-MO. 35-48	0.35	48	0.25	2.8	5