

# VERTICAL ELECTROPHORESIS UNITS

Z33,956-3, Z33,957-1 and Z33,985-7

#### WARNING

THESE UNITS ARE CAPABLE OF DELIVERING POTENTIALLY LETHAL VOLTAGE WHEN CONNECTED TO A POWER SUPPLY AND ARE TO BE OPERATED ONLY BY QUALIFIED TECHNICALLY TRAINED PERSONNEL.

PLEASE READ THE <u>ENTIRE</u> OPERATOR'S MANUAL THOROUGHLY BEFORE OPERATING THIS UNIT.

THESE UNITS COMPLY WITH THE STATUTORY CE SAFETY DIRECTIVES:

73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992 EN 61010-1:1993/BS EN 61010-1:1993

THE SIGMA VERTICAL ELECTROPHORESIS UNITS ARE DESIGNED TO GIVE LONG SERVICE AND REPRODUCIBLE RESULTS IN YOUR LABORATORY. A FEW MOMENTS SPENT READING THESE INSTRUCTIONS WILL ENSURE THAT YOUR EXPECTATIONS ARE REFLECTED IN THE SUCCESSFUL USE OF THE APPARATUS.

FIRST CHECK THAT THE APPARATUS HAS BEEN RECEIVED COMPLETE AND UNDAMAGED FOLLOWING SHIPMENT. ANY FAULTS OR LOSSES MUST BE NOTIFIED TO SIGMA IMMEDIATELY, SIGMA CANNOT ACCEPT RESPONSIBILITY FOR GOODS RETURNED WITHOUT PRIOR NOTIFICATION.

REFER TO THE PACKING LIST AND CHECK THAT ALL COMPONENTS AND ACCESSORIES ARE PRESENT.

PLEASE RETAIN ALL PACKAGING MATERIALS UNTIL THE WARRANTY PERIOD HAS EXPIRED.

#### **SPECIFICATIONS:**

#### Construction:

- Rugged acrylic construction.
- · All acrylic joints chemically bonded.
- Doubly insulated cables, rated safe up to 1,000 volts.
- Gold plated electrical connectors, corrosion-free and rated safe up to 1,000 volts.
- Recessed power connectors, integral with the safety lid.
- 0.2mm diameter platinum electrodes, 99.99% pure.
- User replaceable platinum electrodes.
- Silicone rubber dovetail seal provides leak-free sealing and are easy to clean and or replace.
- User friendly clamping system.
- Wide range of accessories.

#### **Environmental Conditions:**

- This apparatus is intended for indoor use only.
- This apparatus can be operated safely at an altitude of 2,000m.
- The normal operating temperature range is between 4°C and 65°C.
- Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C.
- The apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664. POLLUTION DEGREE 2, states that: "Normally only non-conductive pollution occurs. Occasionally, however, a temporary conductivity caused by condensation must be expected".

# **PACKING LISTS:**

<b>Z33,956-3</b>	10 x 10 cm Vertical Electrophoresis Unit		
		Replacement	
No. Items	<u>Description</u>	Part Number	Check
1	Tank with Safety Lid and cables	-	
1	Gel running module	-	
1	Casting base	Z33,969-5	
2	2.0mm Notched Glass Plates	Z33,959-8	
2	2.0mm Plain Glass Plates	Z33,960-1	
1	Dummy Plate	Z33,962-8	
2	Sets of 1.0mm Spacers	Z33,964-4	
1	Set spacer aligners	-	
2	Combs 1.0mm, 12 Sample	Z33,977-6	
Z33.937-1	TO X TO CITI COOLEG VEHICAL ELECTION	ophoresis onii	
<u>Z33,957-1</u>	10 x 10 cm Cooled Vertical Electron	Replacement	
No. Items	Description	•	<u>Check</u>
		Replacement	<u>Check</u>
No. Items	<u>Description</u>	Replacement	Check
No. Items	Description Tank with Safety Lid and cables	Replacement	Check
No. Items 1 1 1 2	<u>Description</u> Tank with Safety Lid and cables Gel running module	Replacement Part Number	Check
No. Items 1 1 1 2 2	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates	Replacement Part Number 	Check
No. Items  1  1  2  2  1	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates Dummy Plate	Replacement Part Number - - Z33,969-5 Z33,959-8 Z33,960-1 Z33,962-8	Check
No. Items  1  1  2  2  1	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates Dummy Plate Sets of 1.0mm Spacers	Replacement Part Number - - - Z33,969-5 Z33,959-8 Z33,960-1	Check
No. Items  1  1  2  2  1  1	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates Dummy Plate Sets of 1.0mm Spacers Set spacer aligners	Z33,969-5 Z33,969-5 Z33,959-8 Z33,960-1 Z33,962-8 Z33,964-4	Check
No. Items  1  1  2  2  1	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates Dummy Plate Sets of 1.0mm Spacers	Replacement Part Number - - Z33,969-5 Z33,959-8 Z33,960-1 Z33,962-8	Check
No. Items  1  1  2  2  1  1	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates Dummy Plate Sets of 1.0mm Spacers Set spacer aligners	Z33,969-5 Z33,969-5 Z33,959-8 Z33,960-1 Z33,962-8 Z33,964-4	Check
No. Items  1  1  2  2  1  1	Description Tank with Safety Lid and cables Gel running module Casting base 2.0mm Notched Glass Plates 2.0mm Plain Glass Plates Dummy Plate Sets of 1.0mm Spacers Set spacer aligners	Replacement Part Number  Z33,969-5 Z33,959-8 Z33,960-1 Z33,962-8 Z33,964-4 Z33,977-6	Check

		Replacement	
No. Items	<u>Description</u>	Part Number	<b>Check</b>
1	Tank with Safety Lid and cables	-	
1	Gel running module	=	
1	Casting base	Z33,998-9	
2	4.0mm Notched Glass Plates	Z33,986-5	
2	4.0mm Plain Glass Plates	Z33,987-3	
1	Dummy Plate	Z33,988-1	
2	Sets of 1.0mm Spacers	Z33,991-1	
1	Set spacer aligners	-	
2	Combs 1.0mm, 24 Sample	Z34,016-2	

#### **USING THE 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 wellventilated 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 DISTILLED 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 SIGMA-ALDRICH 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

Water cooled units can be stored with water in the base core but 0.02% sodium azide should be added to prevent algal growth. 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 <u>neutral</u> Decon overnight and then flush through with clean water.

## **D. Filling the Base Cooling Core:**

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 deionised water containing 0.02%(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. <u>DO NOT</u> 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

- 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 silverstained 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.

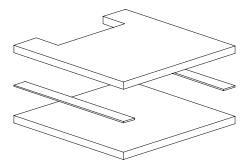
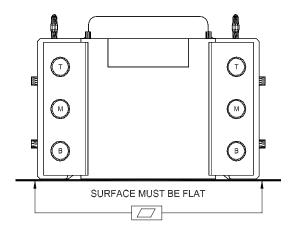


Figure 1.

- 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 SIGMA 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 SIGMA 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. The plates can be kept in position by pressing on the tops of the plate assemblies during tightening. 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 (see fig. 4, page 10).

3. Turn the cams through a maximum of ~170° so that the handles are facing almost vertically upwards, or until finger tight (fig 5). **DO NOT OVER TIGHTEN** past this point as this will only force the glass plates up off the silicone seals.

Figure 2.



#### G. Gel Pouring

- 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. Polymerisation conditions should be adjusted to effect polymerisation 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 450μl of freshly prepared 10% (w/v) Ammonium persulphate plus 200μl TEMED. The setting time increases to about 10 minutes if the TEMED volume is reduced to 100μl and to approximately 15 minutes with 75μl. 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 SIGMA Gel Casting Unit or by clamping a taped gel into the tank unit.
- Run the acrylamide separating gel mix slowly down the inside edge of the gel cassette (fig 6). Avoid aeration. If a stacking gel is to be used, carefully overlay the separating gel to a depth of 3 - 5mm with 1 x separating gel buffer or water-saturated butanol.
- 4. Following polymerisation 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 (see figure 7, page 11). 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.

- 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.
- 2. 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 (fig 8).
- 3. Carefully remove the comb ensuring not to damage the wells (fig 9).
- 4. Add the appropriate volume of running buffer to the upper and lower chambers (see table 1 on page 8), replace the lid and connect to a suitable power supply (fig 10). 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.

#### 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 of 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.

<u>Model</u>	Buffer Volume Approx. Gel		Run (	<u>Conditions</u>
	<u>(ml)</u>	Volume (ml)	<u>Voltage</u>	<u>Current</u>
			(Volts)	(mAmps)
Z33,957-1,	Upper 120	7	150-225	25-45 (1 gel)
Z33,956-3	Lower 1120			50-85 (2 gels)
(10 x 10cm)				
Z33,985-7	Upper 650	35	90-120	(Stacking Gel)
(20 x 20cm)	Lower 3500		120-180	(Resolving Gel)
				20-30 (Per Gel)

- The run conditions are to be taken as a guideline only and apply to SDS Tris-glycine gels. If the plates become hot increase the water flow rates within the recommended limits or reduce the power settings.
- If a native gel is being used, pre-electrophorese the gel for 15 40 minutes prior to loading.
- 3. For SDS 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.
- Replace the safety lid firmly making sure that the electrical connectors form a good contact.
- 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, 1mm gel slot.

#### Table 2.

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

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 minimises 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 SIGMA Gel Casting Unit

## Fig 3

Make sure that the bottom edges of the glass plates and spacers are perfectly aligned. Gel Casting Base turned upside down makes an ideal flat surface for this purpose



## **Fig 4**

Place the assembled Module on the Gel Casting Base, ensuring The that the silicone mats are butting up fully against the bottom of the gel plates



# <u>Fig 5</u>

Push the cam pins in so that they locate in the recesses in the bottom of the Central Module. Turn the levers to pull the Module down tightly onto the silicone mats.



# Fig 6

When locked securely in position, carefully pour the acrylamide between the plates as described in Section G Gel Pouring (page7).



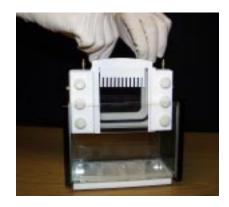
# <u>Fig 7</u>

Before the gel polymerizes, place an appropriate comb between the gel plates and allow to set.



## Fig 8

Release the Central Gel Running Module from the Casting Base by removing the cam pins. Place the Central Gel Running Module into the main buffer tank



# <u>Fig 9</u>

Remove the comb from between the plates taking care not to damage the wells. Note that when running only one gel the blanking plate supplied is required on the other side of the unit to retain the top buffer



# Fig 10

After filling the tank chambers with a necessary volume of buffer and loading the samples into each well, replace the lid and connect the cables to a suitable power supply for electrophoresis.



# **Comb Specifications:**

Code	Thickness mm	No.Samples	Tooth Width mm	Tooth Spacing mm	Sample <u>Volume</u> ( <u>Per Well)</u> μ <u>I</u>
Combs for 10 x 10 c	cm gel systems.				
Z33,972-5 Z33,973-3 Z33,974-1 Z33,976-8 Z33,977-6 Z33,978-4 Z33,979-2	0.75 0.75 0.75 1 1 1	10 12 16 10 12 16 10	4 3.75 2.5 4 3.75 2.5 4	3 2.25 2 3 2.25 2	30 28 18 40 37 25 60
Z33,980-6	1.5	12	3.75	2.25	56
Z33,981-4	1.5	16	2.5	2	37
Z33,982-2	2	10	4	3	80
Z33,983-0	2	12	3.75	2.25	75
Z33,984-9	2	16	2.5	2	50
Combs for 20 x 20 o	cm gel systems. 0.75	10	11	5.5	82
Z34,008-1	0.75	18	6	3	45
Z34,010-3	0.75	24	4.75	2	35
Z34,011-1	0.75	36	2.5	2	18
Z34,013-8	0.75	48	2.35	1	17
Z34,014-6	1	10	11	5.5	110
Z34,015-4	1	18	6	3	60
Z34,016-2	1	24	4.75	2	47
Z34,017-0	1	36	2.5	2	25
Z34,018-9	1	48	2.35	1	23
Z34,019-7	1.5	10	11	5.5	165
Z34,020-0	1.5	18	6	3	90
Z34,021-9	1.5	24	4.75	2	71
Z34,022-7	1.5	36	2.5	2	37
Z34,023-5	1.5	48	2.35	1	35
Z34,024-3 Z34,025-1 Z34,027-8 Z34,028-6	2 2 2 2 2	10 18 24 36	11 6 4.75 2.5	5.5 3 2 2	220 120 95 50
Z34,029-4	2	48	2.35	1	47
Z34,030-8	3	10	11	5.5	330
Z34,031-6	3	18	6	3	180
Z34,032-4	3	24	4.75	2	142.5
Z34,033-2	3	36	2.5	2	75
Z34,034-0	3	48	2.35	1	70

# **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 contact your local SIGMA-ALDRICH office.

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. (No casting base).	Ensure that the sealing surfaces of the glass 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

Problem	Comments
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.

Comments

Problem

#### References

- 1. Maniatis, T., E. F. and Sambrook, 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 8:3, 3.
- 4. Ausubel, et al., (eds). (1993) Current Protocols in Molecular Biology. Greene Publishing and Wiley-Interscience, New York.

# **Related products**

The following Sigma-Aldrich chemical and other consumable products are mentioned in this manual

For up-to-date packs and prices, see <a href="www.sigma-aldrich.com">www.sigma-aldrich.com</a> or contact your local Sigma-Aldrich sales office (see back of manual)

Related Product	Sigma-Aldrich Prod. No.		
Acrylamide	01696		
TEMED	T9281		
Ammonium persulfate	09915		
Dimethyldichlorosilane	D3879		
Silver stain kit for proteins	85181		
Ethidium bromide solution	E1510		

See the Sigma and Fluka catalogues for more buffers, molecular weight markers and other useful electrophoresis products.

# **Notes**

# **Notes**

# **QUALITY CHECK LIST**

Model	Serial Number
<ol> <li>Tank Leak Tested</li> <li>Electrode Conductivity Test</li> <li>Labels Positioned</li> <li>Labels Test/Serial No.</li> <li>Unit Scratch/Blemish Free</li> </ol>	Check  Check  Check  Check  Check
<ul><li>6. Accessories - See Packing List</li><li>7. Instructions</li></ul>	Check
CONTROL PROCEDURES.	UPPLIED HAVING PASSED RIGOROUS QUALITY  RY, PLEASE CONTACT YOUR LOCAL SIGMA-ALD- NICAL SUPPORT.  SIGNED
	QUALITY CONTROL ASSESSOR

# WARRANTY

SIGMA-ALDRICH guarantees that the unit you have received has been thoroughly tested and meets its published specification.

This unit (excluding all accessories) is warranted against defective material and workmanship for a period of twelve (12) months from the date of shipment ex factory.

SIGMA-ALDRICH will repair all defective equipment returned during the warranty period without charge, provided the equipment has been used under normal laboratory conditions and in accordance with the operating limitations and maintenance procedures outlined in this instruction manual and when not having been subject to accident, alteration, misuse or abuse.

No liability is accepted for loss or damage arising from the incorrect use of this unit. SIGMA-ALDRICH's liability is to the repair or replacement of the unit or refund of the purchase price, at SIGMA-ALDRICH's option. SIGMA-ALDRICH is not liable for any consequential damages.

SIGMA-ALDRICH reserves the right to alter the specification of its products without prior notice. This will enable us to implement developments as soon as they arise.

SIGMA-ALDRICH products are for research use only.

A return authorisation must be obtained from SIGMA-ALDRICH before returning any product for warranty repair on a freight prepaid basis.

#### WARNING

DO NOT attempt to remove the outer casing or make repairs to our electrical range of products, should any unit fail.

Contact SIGMA-ALDRICH immediately if the need for repair or servicing should arise.

See back cover for contact details of your local SIGMA-ALDRICH office

# Sigma-Aldrich Sales Offices in Europe

#### Austria

SIGMA-ALDRICH Handels GmbH Hebbelplatz 7 1100 Wien Tel: 01-605 81 10 Fax: 01-605 81 20

E-mail: sigma@sigma.co.at

#### Belgium

SIGMA-ALDRICH nv/sa K.Cardijnplein 8 B-2880 Bornem Tel: 03 899 13 01 Fax: 03 899.13.11 E-mail: sigmaldr@sial.be

## Czech Republic

SIGMA-ALDRICH s.r.o. Pohrezni 46 186 00 Praha 8 Tel: 00-420-2-2176 1300 Fax: 00-420-2-2176 3300

E-mail: CZECustSV@eurnotes.sial.com

#### Denmark

SIGMA-ALDRICH Denmark S/A Vejlegaardsvej 65 B DK-2665 Vallensbaek Strand Tel: +45 43 56 59 00 Fax: +45 43 56 59 05

E-mail: denorder@eurnotes.sial.com

#### Eire

SIGMA-ALDRICH Ireland Ltd Airton Road Tallaght Dublin 24 Tel: 01 404 1900 Fax: 01 404 1910

E-mail: EICustsv@eurnotes.sial.com

#### Finland

SIGMA-ALDRICH Finland YA-Kemia Oy Teerisuonkuja 4 00700 Helsinki Tel: (09) 350-9250 Fax: (09) 350-92555

E-mail: finorder@eurnotes.sial.com

#### France

SIGMA-ALDRICH Chimie S.a.r.I L'Isle D'Abeau Chesnes B.P. 701 38297 St.Quentin Fallavier

Tel Numero Vert: 0800 21 14 08 Fax Numero Vert: 0800 03 10 52 E-mail: fradvsv@eurnotes.sial.com

#### Germany

SIGMA-ALDRICH Chemie GmbH Gruenwalder Weg 30 D-82041 Deisenhofen Tel: +49(0)89-6513-0 Fax: +49(0)89-6513-1169

E-mail: DeOrders@eurnotes.sial.com

#### Greece

SIGMA-ALDRICH (o.m) Ltd 72 Argonafton Str 16346 Ilioupoli, Athens Tel: 30-1-9948010 Fax: 30-1-9943831

E-mail: GRCustSV@SIALEUROPE

## <u>Hungary</u>

SIGMA-ALDRICH Kft 1399 Budapest Pf. 701/400 Magyarorszag Tel: 06-1-235-9055 Fax: 06-1-235-9050 E-mail: info@sigma.sial.hu

#### Italy

SIGMA-ALDRICH S.r.I. Via Gallarate 154 20151 Milano Tel: 02-33417.310 Fax: 02-38010.737

E-mail: itorder@eurnotes.sial.com

#### <u>Netherlands</u>

SIGMA-ALDRICH Chemie BV Stationsplein 4 E Postbus 27 NL-3330 AA Zwijndrecht Tel: 078-620 54 11 Fax: 078-620 54 21 E-mail: nlcustsv@eurnotes.sial.com

#### Norway

SIGMA-ALDRICH Norway AS PO Box 4297 - Torshov Sandakerveien 102 N-0401 OSLO Tel: 47 22 091500 Fax: 47 22 091510

E-mail: nororder@eurnotes.sial.com

#### Poland

SIGMA-ALDRICH Sp.zo.o Szelagowska 30 61-626 Poznan Tel: (48-61) 823-24-81 Fax: (48-61) 823-27-81 E-mail: plcustsv@eurnotes.sial.com

#### **Portugal**

SIGMA-ALDRICH Quimica S.A. (Sucursal em Portugal) Aptdo. 131 2710 Sintra Free phone: 0800-202180 Free fax: 0800-202178 F-mail

encomendas: poorders@eurnotes.sial.com

#### Spain

SIGMA-ALDRICH Quimica S.A. Aptdo, 161 28100 Alcobendas (Madrid) Tel: 91-6619977 Fax: 91-6619642

E-mail, pedidos: esorders@eurnotes.sial.com

#### Sweden

SIGMA-ALDRICH Sweden AB Solkraftsvagen 14C 135 70 Stockholm Tel: 020-35 05 10 Fax: 020-35 25 22

E-mail: sweorder@eurnotes.sial.com

#### Switzerland

SIGMA-ALDRICH Chemie A division of Fluka Chemie AG Postfach 260 CH-9471 Buchs Tel: 081-755 27 21 Fax: 081-755 28 40 E-mail: Fluka@sial.com

#### United Kingdom

SIGMA-ALDRICH Company Ltd The Old Brickyard, New Road Gillingham, Dorset SP8 4XT Tel: 01747 833000

Fax: 01202 715460

E-mail: ukcustsv@eurnotes.sial.com