SIGMA-ALDRICH

CAPILLARY ELECTROPHORESIS UNITS

Z34,001-4 Capillary Electrophoresis set
Z34,002-2 Capillary Electrophoresis Module

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 ENTIRE 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 CAPILLARY 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:

Z34,002-2 is an IEF module, designed for use with the tank, safety lid and cables from the Sigma Cooled Mini Vertical Gel Electrophoresis set.

Z34,047-2 is a ready-to-use set of IEF module, capillary tubes, blanking stoppers, and extraction platform, tank, safety lid and cables. It also contains 1.5mm spacers and sample combs for the size determination (slab gel) phase.

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.

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 4^0C and 65^0C.
- Maximum relative humidity 80% for temperatures up to 31^0C decreasing linearly to 50% relative humidity at 40^0C.

- 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 LIST for Z340014

No. Items	Description	Replacement Part Number	Check
1	Tank with Safety Lid & cables	-	
1	Internal Running Module	Z340022	
1	80mm Capillary Tubes, PK/10	Z340030	
1	Blanking Ports, PK/10	Z340049	
1	Extraction platform	Z340057	
2	1.5mm combs (sample + ref.)	Z340065	
2	sets of 1.5mm spacers	Z339652	

USING THE CAPILLARY 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 central white plastic bar 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 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 neutral 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. 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. Capillary Tube Preparation

- Clean the outside of the tubes in a mild laboratory detergent. A piece of wire with a cotton wool plug placed over the end can be used to clean the insides of the tubes.
- **DO NOT** use abrasive creams or scourers. **NEVER** allow organic solvents or chromic acid to come into contact with the plastic components.
- Handle clean tubes with gloved hands (remove any fingerprints with acetone).

F. Capillary Tube Gel Pouring

1. Tube gels can be poured either by sealing one end of the tube and injecting with acrylamide solution or by placing the tubes in a beaker containing acrylamide solution and then allowing the tubes to fill by capillary action.
2. If sealing is the method of choice, prepare the following solution. This will be enough to pour 20, 80mm Capillary Tubes (Z340030). For Native IEF Gels, do not use Urea and NP-40 and use 18ml of distilled water instead of 16ml;

   16ml Distilled Water (18ml for Native Gels)
   2.4ml Glycerol
   0.9ml 4-8 Resolyte or other commercially available 40% ampholyte solution
   3.8ml Acrylamide/Bis solution
   15µl TEMED
   16.2g Urea (omit for Native Gels)
   0.6ml NP40 (omit for Native Gels)
   We recommend degassing of this solution prior to pouring.
   When ready to pour, add 120µl of 10% w/v ammonium persulphate solution.

3. Seal one end of the tube with Parafilm and place the sealed end facing downwards in the slots of the Capillary Electrophoresis Unit Internal Running Module. To prevent the Parafilm seal from being disturbed when inserting into the unit, insert the top of the tube from the bottom upwards. Alternatively, the bottom ends of the tubes can be sealed when already in the Internal Running Module.
4. Fill a long, narrow bore syringe with acrylamide solution and insert into the capillary tube to within about 1cm of the bottom of the tube, making sure the end of the syringe does not come into contact with the Parafilm seal.
5. Inject the solution slowly, whilst slowly withdrawing the syringe as the solution rises up the tube, always keeping the gap between the end of the syringe and the rising acrylamide solution at about 1cm.
6. When the rising solution has reached to within 1cm of the top, stop filling the tube. The 1cm space left over will act as the sample well. Fill the remaining 1cm gap with water saturated isobutanol.
7. Leave to polymerise, which will normally take 1 – 2 hours.
8. After polymerisation, remove the water-saturated isobutanol. Tube gels can be used immediately or stored wrapped in a damp paper towel and Clingfilm at 4°C. **IMPORTANT: Before electrophoresis, remove the Parafilm from the bottoms of the tubes.**

9. If capillary action is the pouring method of choice, prepare double the amount of acrylamide solution to that in the recipe above. Pipette ~70% of the acrylamide solution into a flat-bottomed beaker and stand the capillary tubes upright in the acrylamide solution. Allow the tubes to equilibrate with the acrylamide solution. Check the height of the acrylamide in the tubes. If the tubes are full so that there is less than a1cm non-filled space at the top, remove some of the acrylamide solution from the beaker until the height is 1 cm from the top. If there is a greater than1cm space at the top, add more acrylamide solution, so that the solution rises in the tubes until there is a 1cm space at the top. Fill the 1cm space, which will act as the sample well, with water saturated isobutanol.
10. Leave to polymerise which will normally take 1 – 2 hours.
11. After polymerisation, remove the water saturated isobutanol
12. The tube gels can now be removed from the beaker and inserted into the slots in the Internal Running Module with the 1cm sample well facing upwards. Alternatively, they can be stored wrapped in a damp paper towel and Clingfilm at 4°C.
    The tubes may contain a residual of acrylamide on the outside and may need cleaning with distilled water before insertion.

G. 1st Dimension (IEF) Phase

Buffer and run conditions will vary according to the type of ampholyte used. The following conditions are given as guidelines only and apply when 4-8 Resolyte is the ampholyte used. Other Ampholytes will require different buffer solutions. Please consult your laboratory manuals.

1. Prepare ~ 500ml of 10mM H_3PO_4 Anode Buffer and use this to fill the bottom chamber of the unit so that the bottoms of the capillary tubes are submerged. If less than 10 capillary tubes are to be run, block up the unused tube slots in the internal running module with the blanking ports provided.
2. Place the Internal running Module into the unit and fill the upper buffer reservoir with ~200 mls of 20mM NaOH Cathode Buffer so that the tops of the capillary tubes are submerged. See Table 1. For a summary of running conditions.

Table 1

Operational:

	Lower Running Buffer	Upper Running Buffer	Max Voltage	Max Current (Per Gel)
Z340014	**500mls**	**100mls**	**1000V**	**110mA**

3. For the Prefocus, load the gels with 10µl of 1% ampholyte solution and run for 15 minutes at 200V, then for 30 minutes at 300 V and then finally 30 minutes at 400V. The Prefocus stage is recommended as it helps set up the pH gradient.

4. Load the tubes with the samples. These should be dissolved in 1% ampholyte with 20% glycerol.
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.
7. Run at 400V for 3 hours and then 800V for 30 minutes.
8. At the end of the run, turn the power supply settings to zero, turn off the mains supply and disconnect the power leads.
9. 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.
10. Remove the Internal Module and remove the tubes from their slots. The gels can be extracted from the capillary tubes by: **a)** inserting a piece of wire with a small plug of cotton wool on the end and using this as a piston to push the gel out, **b)** inserting a Gilson tip into the end of the gel and gently squeezing the gel out with air or water. Whichever of these two methods is used, the gels should be handled with care as they are fragile.

H. 2-D, Size Determination Phase.

1. To prepare the tube gel(s) for the 2-D, size-determining phase, equilibrate them by soaking for 30 minutes in the running buffer to be used for the 2-D phase.
2. Remove the gel(s) from the running buffer pre-soak, and place each lengthways onto the top of a pre-poured slab gel. The slab gel should be casted using a blank or 2-D comb. For details on the casting of slab gels and the use of the SIGMA Vertical Gel Electrophoresis Units, consult the manual accompanying that product.
3. Hold the tube gel in place by pouring over it a low % agarose gel containing the tracker dye.
4. Electrophorese as usual for slab gels until the tracker dye has advanced the required distance down the gel.
5. The samples can be visualized using any of the standard staining methods or can be blotted.

I. At the End of the Run

- Tubes can be cleaned using a mild detergent and rinsing in distilled water. A clean sheet of foam rubber placed at the bottom of the sink serves as a tube support and minimises the risk of tube damage.
- Empty the tank 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.

Related products

The following Sigma-Aldrich chemical and other consumable products are mentioned in this manual
For up-to-date packs and prices, see www.sigma-aldrich.com or contact your local Sigma-Aldrich sales office (see back of manual)

Related Product	Sigma-Aldrich Prod. No.
Acrylamide	01696
Bis	66669
Glycerol	G8773
TEMED	T9281
Ammonium persulfate	09915
Ampholyte solution pH 5.0 – 8.0	10049
Urea	U5378
Tergitol NP40	NP-40
Parafilm	Z10,811-1

See the Sigma catalogue for a wide range of IEF markers.

See the Fluka catalogue for a wide range of Ampholyte solutions.

NOTES

QUALITY CHECK LIST

Model..... Serial Number.....

1. Tank Leak Tested Check.....
2. Electrode Conductivity Test Check.....
3. Labels Positioned Check.....
4. Labels Test/Serial No. Check.....
5. Unit Scratch/Blemish Free Check.....
6. Accessories - See Packing List Check.....
7. Instructions Check.....

ALL SIGMA PRODUCTS ARE SUPPLIED HAVING PASSED RIGOROUS QUALITY CONTROL PROCEDURES.
IF HOWEVER, YOU HAVE A QUERY, PLEASE CONTACT YOUR LOCAL SIGMA-ALDRICH SALES OFFICE FOR TECHNICAL 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 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

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