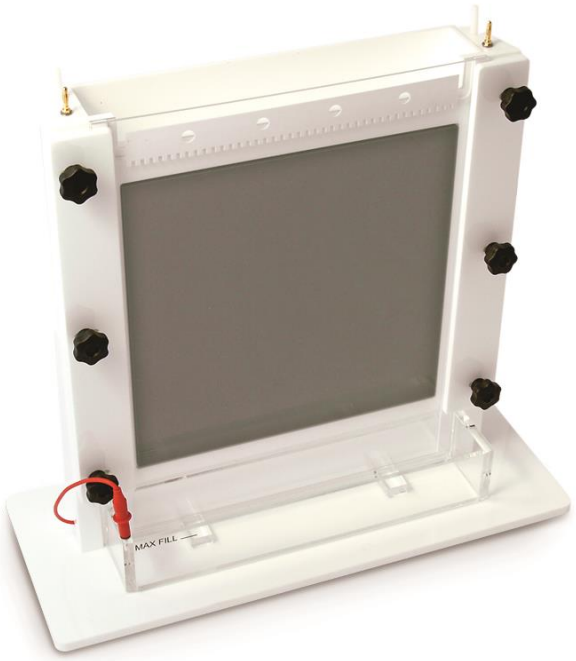
DNA Sequencing Electrophoresis Chamber

AE28.1



WARNING:
Please read the entire operator's manual thoroughly before operating this unit.

Our DNA-sequencing electrophoresis chambers comply with the statutory CE safety directives.

Our DNA-sequencing electrophoresis chambers are designed to give long service and reproducible results in your laboratory. Please take a few minutes to read these instructions to ensure that you will receive full satisfaction.

First check that the apparatus has been received complete and undamaged after shipment. We must be informed immediately of any faults or damages. We will not accept liability for any products returned to us without prior notice.

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

1. PACKING LIST

No. Items	Description
1	DNA Sequencing unit (chamber with safety lid + integral power leads, 2 mm plugs)
1 pair	Electrophoresis cables
1	Plain glass plate
1	Notched glass plate
2	0.35 mm spacers
1	Shark tooth comb, 0.35 mm, for 48 samples

2. ACCESSORIES

Cooling and heating sensor kit	(1 kit)	N705.1
Standard glass plates (330 x 450 mm)	(1 pair)	N707.1
Notched glass plates (330 x 450 mm)	(1 pair)	N706.1
Spacers (0.35 mm)	(1 pair)	N709.1

Spacers:

Thickness (mm)	Width (mm)	Length (mm)	Art. No.	Pack Qty.
0.25	20	450	N708.1	1 pair
0.35	20	450	N709.1	
1.0	20	450	N710.1	
1.5	20	450	AE39.1	

Combs with square wells:

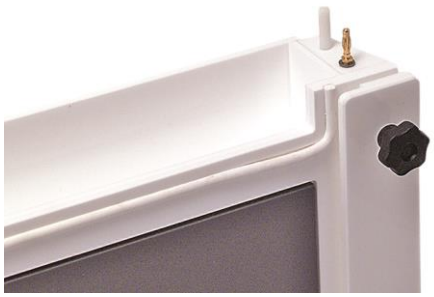
Wells	48		80	
Thickness (mm)	Art. No.	Vol. (µl) for 5 mm thick gel	Art. No.	Vol. (µl) for 5 mm thick gel
1.0	AE31.1	35	AE35.1	20
1.5	AE32.1	50	AE36.1	30
Pack Qty.	1 piece		1 piece	

Shark tooth combs:

Wells	48		96	
Thickness (mm)	Art. No.	Vol. (µl) for 5 mm thick gel	Art. No.	Vol. (µl) for 5 mm thick gel
0.25	N719.1	7	N721.1	3
0.35	N722.1	9	N724.1	5
Pack Qty.	1 piece		1 piece	

3. SPECIFICATION

A. Design



- Rugged acrylic design.
- All acrylic joints chemically bonded.
- Doubly insulated cables, rated safe up to 1.500 volts.
- Gold-plated electrical connectors, corrosion-free and rated safe up to 1.500 volts.
- Recessed power connectors, integrated in the safety lid.
- Replaceable platinum electrodes Ø 0,2 mm, 99.99 %. pure.
- Silicone rubber dovetail seal provides leak-free sealing - easy to clean and or replace.
- User friendly clamping system with screws
- Removable lower buffer tank for easy buffer disposal and cleaning.
- Drainage port on the upper buffer tank for easy buffer disposal.
- Wide range of accessories.

B. Environnemental Conditions

- This apparatus is intended for indoor use only.
- This apparatus can be operated safely up to an altitude of 2,000 m above sea level.
- The normal operating temperature range lies between 4 °C and 65 °C.
- The device is designed for a relative humidity of max. 80 % at temperatures up to 31 °C. At temperatures above 31 °C up to max. 40 °C a relative humidity of up to 50 % is permitted - with linear decrease.
- 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, temporary conductivity caused by condensation must be expected”.

C. Operational Conditions

Running Buffer Volume (ml)	Maximum Voltage (V)	Maximum Current (mA)
Upper: 400-1,000	1,500	100
Lower: 400-1,000	1,500	100

Important: Do not fill over the maximum fill lines!

4. USING VERTICAL DNA SEQUENCING UNIT

A. Safety Precautions

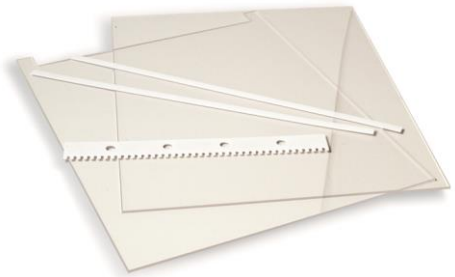
- Please READ the instructions before using the apparatus.
- Always isolate the electrophoresis units from their power supply before removing the safety lid. Isolate the power supply from the mains FIRST then disconnect the leads.
- DO NOT exceed the maximum operating voltage or current (see Specifications).
- DO NOT operate the electrophoresis units in metal trays.
- Acrylamide is a volatile, cumulative neurotoxin and suspected carcinogen. Always wear effective protective clothing and follow the recommended handling and disposal procedures.
- Polymerised gels contain some unpolymerised monomer. Only handle with gloves.
- After the replacement of a platinum electrode have the unit inspected and approved by your safety officer prior to use.
- DO NOT fill the chambers 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 used. 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 down on the pegs located on the top of the unit with your thumbs and lift the lid vertically with your fingers.
- Before use clean the apparatus with DISTILLED WATER ONLY. Dry the unit carefully. IMPORTANT: Acrylic plastic is NOT resistant to aromatic or halogenated hydrocarbons, ketones, esters, alcohols (over 25 %) or acids (over 25 %).
- Before use, and then on a monthly basis, check the unit for any leaks at the chemically bonded joints. Place the unit on a sheet of dry tissue paper and then fill with DISTILLED WATER ONLY to the maximum fill line. Any leakage will be seen on the tissue. If any leakage is seen DO NOT ATTEMPT TO REPAIR OR USE THE APPARATUS. Notify Carl Roth GmbH + Co. KG (0721/5605-172) 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 thorough rinsing with distilled water should be sufficient.
- Ensure that the connectors are clean and dry before usage or storage.

C. Preparation of Glass Plates

- All glass plates - new and used - **must be cleaned thoroughly** before use, as there are always impurities on the surface such as grease stains, fingerprints or gel particles that can affect the gel. Remove most of the contamination using a neutral detergent and a nail brush. Do not use metal handled test tube brushes, abrasive cleaning creams or scourers as these may scratch and impair the performance of the products. You may find that a sheet of clean foam rubber serves as a useful support during washing to minimise the risk of plate damage. **Rinse and dry** the plates as follows: deionised water - ethanol – acetone- ethanol – deionised water.
  DO NOT ALLOW ACETONE OR OTHER ORGANIC SOLVENTS TO COME INTO CONTACT WITH THE PLASTIC COMPONENTS.
- Although not readily visible there is often residual grease which should be removed with a tissue soaked in chloroform or dichloroethane in a fume hood. When clean, treat the plates as described below.
- To ensure that the gel can be easily removed later, it is advisable to **siliconise** *the notched glass plate* with a tissue soaked in dimethyldichlorosilane. Wipe the plate, including the lugs, in a fume hood. Rinse with deionised water and dry with a tissue.
- *The plain glass plate* should be siliconised ONLY 1 cm length-wise along the edge i.e. along where the spacers lie.
- If both glass plates are siliconised you may experience difficulty when pouring gels horizontally. Should this situation arise by mistake you can try to remove the siliconising agent with the solvent in which the reagent was supplied, e.g. dimethyldichlorosilane is usually supplied in dichloroethane



- The siliconising treatment is normally sufficient for several gels, but thorough cleaning – as described above – is required after each application. As this can damage the silicone layer, we recommend that you also carry out the siliconisation after each application. This always ensures that the gel can be easily removed from the glass plate. Routine cleaning simply consists of removing all particles of gel with a nail brush and neutral detergent. Clean thoroughly and rinse with deionised water.

D. Reagent Preparation and Gel Volumes

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.

Polymerisation conditions should be adjusted to effect polymerisation within about 5-15 minutes. Test a small volume in a vial prior to pouring the gel. As a rough guide 100 ml degassed 6 % acrylamide gel will set in about 5 minutes at room temperature when gently mixed with 450 µl freshly prepared 10 % (w/v) ammonium persulphate plus 200 µl TEMED. The setting time increases to about 10 minutes if the TEMED volume is reduced to 100 µl and to approximately 15 minutes if it is reduced to 75 µl. The amount of TEMED may need to be reduced at higher room temperatures. **Do not pour under direct sunlight.**

Gel volume

For a 1 mm thick gel 125 ml gel solution is required. For gels of other thickness this amount is multiplied by the corresponding spacer diameter.

Separation area

The pore size of the gel depends on the acrylamide concentration. This results in different separation ranges for proteins (see table below):

Acrylamide Percentage	Separation Resolution
5 %	20 – 220 kD
7,5 %	30 – 120 kD
10 %	20 – 75 kD
12 %	17 – 65 kD
15 %	15 – 45 kD
17,5 %	12 – 30 kD

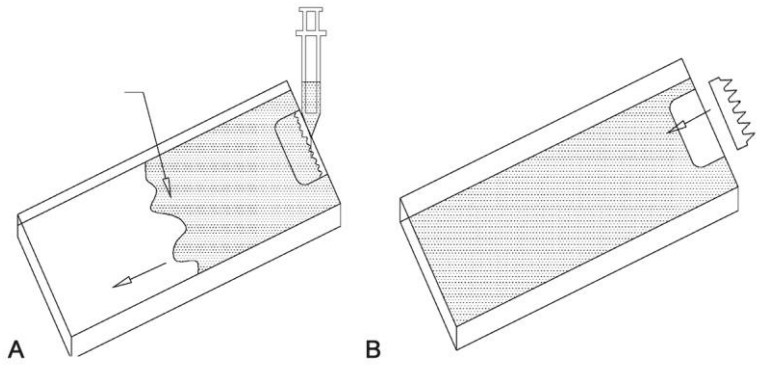
E. Gel Pouring

- **Preparation**
  Remove any greasy fingerprints or water on the edges of the glass plates by wiping with acetone. Lay the plain glass plate on a flat surface, paper towels are suitable as base. Arrange the spacers flush with the edges of the plates. Carefully lay the notched glass plate on top and clamp the plates together (e.g. with clamps Art. No. 0850.1). The gel sandwich is now ready for pouring.

  Please note: For gels thicker than 0.35 mm, the glass plate sandwich must also be carefully sealed at the bottom end with insulating tape.

  Fill a syringe without air bubbles with the desired amount of gel (see "Gel volume").

- **Horizontal pouring**
  Lay the clamped plates horizontally on paper tissues. Hold the syringe as vertically as possible and allow the gel mix to flow from the syringe into the notched area (see Figure A).



  Move the syringe slowly from one side of the notched area to the other. The gel mix should form a continuous pool along the top of the gel space and by capillary action it will move down between the plates. Do not overfill the notched area - the pool should be approximately half the height of the glass lugs. Alternatively, do not let the pool run dry. The gel plates can be tapped slightly behind the moving gel boundary with your knuckles to prevent any bubble formation. When the gel boundary reaches the bottom of the glass plates, carefully reduce the gel flow. Failure to do so will result in the gel mix dripping from the bottom end. Always remove (e.g. with tissue paper) and dispose of any excess gel expertly.

- **Alternative: Diagonal pouring**
  An alternative to horizontal pouring is to pour diagonally. After clamping the plates together, seal the bottom end tight with leak-proof sticky tape (packing tape, textile tape) not forgetting the corners. Hold the glass plates diagonally. Attach a thick cannula onto the syringe and **slowly** press the gel solution into the middle of the notched area between the plates. The solution will flow down the middle to the bottom and then rise slowly towards the notched area. Bubble formation can be prevented by tapping the sides gently with your knuckles. When the solution has almost reached the notched are, carefully reduce the gel flow to prevent the solution from overflowing. After pouring, lay the gel horizontally or slightly diagonally with the notched area face up on some tissues.

- **Inserting the comb**
  Remove any surplus gel from notched area and carefully insert the comb required. If you are using a comb with square wells insert the teeth without trapping bubbles. If you are using a shark tooth comb insert the flat face of the comb at a slight angle to prevent bubbles (see figure B). Add a few drops of gel mix if necessary. Carefully straighten the comb so that it is parallel to the top of the gel plate and reaches 3-5 mm below the notched area.

- **Gel polymerisation**
  The polymerisation should be completed within 10 minutes but allow the gel to polymerise further for at least 90 minutes. If the gel is to polymerise overnight, enclose wet tissues under a cling film seal to prevent the ends of the gel from drying out.

F. Unit Assembly

- Place the safety lid on the device and check the electrical alignment of the connections, black is negative, red is positive.
- Replace the safety lid and attach the electrical connectors to the buffer chamber.
  Please note: Never attach the cables when the lid is on the device! Otherwise the gold-plated terminals will be loosened, which will damage the electrodes.
- Slide the lower buffer chamber into position. Attach the connecting cable to the upper buffer chamber.
- Remove any clamps and the bottom tape, if used.
- The shark tooth comb should be removed if used. However, please leave the square tooth comb in the gel.
- Loosen the screws and push the glass plate sandwich behind the clamping bars. Carefully and evenly tighten the screws.

  Please note: Do not tighten the screws too much, in order to prevent formation of tension in the gel during heating.

G. Running Conditions and Buffer Volume

- Make sure that the upper buffer tank drainage tube is tightly closed.
- Pour app. 800 ml electrophoresis running buffer into the upper and lower buffer chambers. IMPORTANT do NOT fill over the maximum fill lines.
- Ensure that there is no buffer leakage.
- If you are *using a square tooth comb*, remove it carefully. Rinse the wells with electrophoresis buffer.
  If you are *using a shark tooth comb*, rinse the upright well with electrophoresis buffer. Now insert the comb carefully, teeth first, into the gel so that the tips are just inserted in the gel.
- If desired pre-run the gel until the glass plates are warm. Use the settings described below.

H. Sample loading and gel run

- Prior to loading your samples flush out the wells with running buffer to clear them of urea.
- *DNA samples:* The loading volume depends on the capacity of the gel pockets (see above). Fill the sequencing samples in micro centrifuge tubes and heat them at 95 °C for 3 minutes, place on ice and centrifuge - 12,000 x g for 3 minutes. Return to ice.
- *Protein samples (denatured):* The loading volume depends on the capacity of the gel pockets (see above). Fill the sequencing samples in micro centrifuge tubes and add the appropriate amount of 4 x concentrated gel loading buffer. It is recommended to use an additional protein marker, which is also prepared accordingly. Heat the samples for 2 minutes in a water bath or heating block to denature them. Then centrifuge them for 20 seconds at 12,000 rpm.
- Load the gel wells with the desired amount of sample solution. If possible, avoid taking the sample from the bottom of the tube - particulate materials may cause streaking or smearing. You can prevent the sample from running off by applying them in a thin layer directly to the bottom of the well.
- Place the safety lid on the chamber. Make sure that the lid completely covers the electrical connectors.
- Connect and run the gel at the desired power setting (e.g. power supply A541.1). **The leads and electrical connectors are tested to 1,500 volts** and users are advised not to exceed this voltage.
- Good results are obtained for DNA sequencing gels at a constant power of 45-55 Watt with passive ventilation.
  If the *cooling/heating sensor kit* is used the power can be increased up to 75 watts. Ensure that the voltage does not exceed the maximum recommended voltage. The resistance of the gel changes throughout electrophoresis when run at constant power so the starting and finishing voltages will be different.
- In general, the gel temperature should reach 50-60 ^{0}C. At temperatures above this range there is the risk of generating smeared bands or damaging the glass plates.

I. Ending the Run

- Isolate the power supply before removing the connectors.
- Remove the safety lid by gripping the edges and pushing down on the pegs located on the top of the unit with your thumbs.
- Disconnect the leads on the upper and lower buffer chambers.
- Open the upper buffer tank drainage tube. The buffer will then flow into the bottom tank.
  IMPORTANT: Make sure that the bottom tank does not overflow.
- Remove the lower buffer chamber and carefully dispose of the buffer.
- Dismantle the gel sandwich and separate the plates with a strong, thin, broad blade.
  Usually the gel sticks to the non-siliconised plate.
  CAUTION: Do not separate the glass plates at the lugs. Concentrated leverage in any one area may damage the plates.
- *DNA gel:* Roll a strong piece of Whatman paper onto the gel and gently press it all over the gel, especially around the edges. Then slowly pull the gel, which is now sticking to the Whatman paper, from the glass plate and dry it on a vacuum drier.
- *Protein gel:* Moisten the gel with buffer solution and carefully remove it with a spatula. It can then be further processed as required: Coomassie or silver staining, electro blotting, etc.
- The glass plates and buffer chambers are then cleaned as described above.
- Dry the electrical connectors thoroughly with a tissue before storing the unit.

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