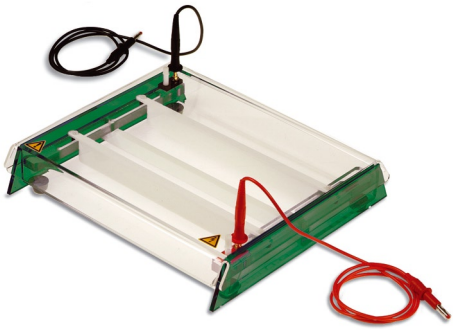
Consort

MANUAL



E3700

July 2009

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CONSORT 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.

CONSORT 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. **CONSORT**'s liability is limited to the repair or replacement of the unit or refund of the purchase price, at **CONSORT**'s option. **CONSORT** is not liable for any consequential damages.

CONSORT 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.

CONSORT products are for research use only.

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

- When used correctly, these units pose no health risk.
- However, these units can deliver dangerous levels of electricity and are to be operated **only** by qualified personnel following the guidelines laid out in this manual.
- Anyone intending to use this equipment should **read** the complete manual thoroughly.
- The unit must **never** be used without the safety lid correctly in position.
- The unit should not be used if there is any sign of **damage** to the external tank or lid.
- 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.
- **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.

- Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60°C can cause damage to the unit and components.** The tank should be thoroughly rinsed with warm water or distilled water to prevent build up of salts but care should be taken not to damage the enclosed electrode and vigorous cleaning is not necessary or advised. Air drying is preferably before use.
- The units should only be cleaned with the following: warm water with a mild concentration of soap or other mild detergent (compatible detergents include dish washing liquid, hexane and aliphatic hydrocarbons). The units should not be left to in detergents for more than 30 minutes.
- **The units should never come into contact with the following cleaning agents, these will cause irreversible and accumulative damage:** acetone, phenol, chloroform, carbon tetrachloride, methanol, ethanol, isopropyl alcohol alkalis.
- **In case of Rnase Decontamination** clean the units with a mild detergent as described above. Wash with 3 % hydrogen peroxide (H_2O_2) for 10 minutes. Rinsed with 0.1 % DEPC- (diethyl pyrocarbonate) treated distilled water (**Caution:** DEPC is a suspected carcinogen. Always take the necessary precautions when using.) RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Environmental conditions

- This apparatus is intended for indoor use only.
- This apparatus can be operated safely at an altitude up to 2000 m.
- 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”.

Fitting electrode cables

1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables, black is negative and red positive.
2. Remove the lid from the unit. Note if the lid is not removed, fitting the cables may result in un-tightening of the gold plug and damage to the electrode.
3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

Solutions

Running buffer

- Tris hippurate 0.05 M, 8.8 pH
- Barbital tris 0.05 M

The following protocol is guideline only.

Please, follow the manufacturers recommended instructions with the types of cellulose acetate strip and samples being used.

1. Position the wick carrying bars in the tank such that the distance between them is just longer then the strip to be used when applied lengthways.
2. Cut to the appropriate size and position the electrode wicks in the tank so that they overhang the wick carrying bars with the base sitting in the buffer tanks. Filter paper or paper towels can be used as the wicks.
3. Equilibrate the strip in buffer with agitation for 10...15 minutes.
4. Fill the tank with 180 ml of buffer per chamber side (360 ml in total).
5. Holding the strip by the edges only in gloved hands, blot the strip in between two pieces of filter paper.
6. Place the strip on top of the wicks and between the bars with the sample end at the negative electrode. Make sure there is good contact by pushing down on the ends of the strip. Glass sliders can be placed over each end to further ensure a good contact.
7. Apply the samples at the negative end of the cellulose acetate plate. Usually a small amount of dye can also be loaded to indicate sample migration distance.
8. Place the safety lid onto the tank and connect the leads to a power supply.
9. Run the electrophoresis at 200 V for 10...15 minutes. Once the electrophoresis is complete, the strip can be stained.

Staining

1. Immerse the strips in about 50 ml of Ponceau S staining solution for 5...10 minutes (amidoblack may be used if prefered) with agitation.
2. Remove Ponceau S and destain with 3 baths of 5% acetic acid for 3 minutes each.

Equipment problem

- *Bubbles do not appear on the electrodes:*

  Ensure that the power supply and the whole electric assembly is operating properly.

- *Melted agarose leaks when casting:*

  Ensure the agarose is not too hot when poured.

  Ensure that the sealing surfaces of the running tray and the gel casting gates are clean.

  Ensure that the ends of the running tray are flat and free of nicks.

Electrophoresis problem

- *Sample well deformed:*

  Allow the gel to set for a minimum of 30 minutes.

  Leave comb in position until gel returns to room temperature before removing.

  Remove the comb both slowly and at a slight angle to prevent gel from breaking.

  Avoid damaging the well with the pipette when loading the sample. Aim for the centre of the well and avoid damaging the bottom of the well with the pipette tip.

- *Samples leak underneath the gel upon loading:*

  The bottom of the wells were torn when the comb was removed. To avoid this tearing, carefully wiggle the comb to free the teeth from the gel.

- *Distorted sample wells:*

  Incomplete polymerisation produces poorly defined wells. De-gas gel solution prior to casting and increase ammonium persulphate and TEMED concentrations.

- *Samples do not run straight:*

  Comb may be warped and should be replaced.

  Running tray may be warped and should be replaced. Reduce the voltage.

  Choose a buffer with suitable ionic strength and buffering capacity.

- *"Smiling" along one edge of the gel:*

  Gel was not level when cast or run. Use a gel levelling table to ensure that the apparatus is level prior to gel casting and electrophoresis.

- *Bromophenol blue dye turns yellow:*

  Check pH of buffer during electrophoresis. (pH change).

  Ensure Tris base and not Tris-HCl was used.

  Mix the buffer periodically during electrophoresis. Connect a pump to circulate the buffer.
- *Double-banded pattern*

  Ensure the comb is vertical during casting so that the well shape is not distorted.

  Decrease the buffer level to 1 mm above the top of the gel. This will reduce the temperature gradient through the gel.

  Increase concentration of the sample and use a thin (2 to 3 mm) gel with a thin (1 mm) comb.
- *"Tailed" bands (excessive fluorescence appearing above the band)*

  Reduce DNA in the sample.

  Reduce the protein and/or glycerol in the sample.
- *Poor band resolution*

  Add ficoll, glycerol, or sucrose to the sample loading buffer to ensure that the sample layers on the bottom of the well. Ensure sample is completely dissolved.

  Reduce voltage, sample concentration, or sample volume.

  Ensure there is at least 1 mm of gel below the bottom of the comb to prevent samples from leaking out the bottom of the well.

  Reduce salt concentration of the sample. High salt concentrations can cause "pinched" lanes, smeared lanes, arched dye front and slow migration.

  Check enzyme activity as it may require longer digestion or different restriction buffer.

  Prepare fresh sample if nuclease contamination is suspected.

  Choose agarose with low endosmosis value.
- *Gel melts or softens near sample wells.*

  Caused by a combination of pH drift and high temperature. Circulate or remix buffer periodically or reduce the voltage.

DECLARATION OF CONFORMITY

We declare under our sole responsibility that the product

Electrophoresis Apparatus
content of the type numbers

E3700

to which this declaration relates is in conformity
with the following standards

EN61010
LOW VOLTAGE DIRECTIVE 73/23/EEG
EN50081-1
EN50082-1
EN60555-2
EMC DIRECTIVE 89/336/EEG

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The electro-magnetic susceptibility has been chosen at a level that gains proper operation in residential areas, on business and light industrial premises and on small-scale enterprises, inside as well as outside of buildings. All places of operation are characterised by their connection to the public low voltage power supply system.

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www.consort.be

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