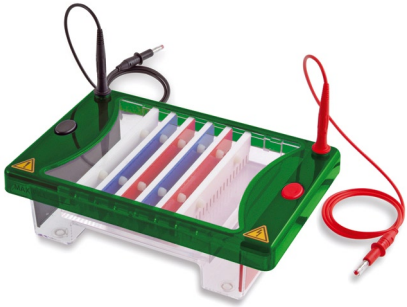
Consort

MANUAL



E3100
E3200
E3300
E3400
E3500
E3600

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.

Fitting loading guides

These can be fitted to enhance visibility of the wells if desired. They can be fitted to the white vinyl platform sheet or to the unit itself.

1. Seat the tray in the unit and note the position of the comb grooves. The samples run black to red but the trays can be used frontward or backwards so ensure that the comb grooves closest to the black electrode are marked.
2. Remove the tray.
3. Peel the back off of the loading guide and carefully apply the loading guide directly to the gel platform.

1. Table below shows the volume of agarose solution required to make the desired agarose gel (5 mm thick) for each unit tray size. For a standard 0.7 % agarose gel, add 0.7 g of agarose to 100 ml of 1x TAE or TBE solution. The same 1x solution should be used in the tank buffer solution.

Model	Gel size (cm)	Volume (ml)
E3100	10x8	40
E3200	7x7	25
	7x10	35
E3300	10x7	35
	10x10	50
E3400	15x7	53
	15x10	75
	15x15	113
E3500	20x10	100
	20x20	200
E3600	26x16	208
	26x24	312
	26x32	416

2. Add the agarose powder to a conical flask.
3. Add the appropriate amount of 1 x TAE or TBE solution from the table above. To prevent evaporation during the dissolving steps below, the conical flask should be covered with para-film.
4. Dissolve the agarose powder by heating the agarose either on a magnetic hot plate with stirring bar or in a microwave oven. If using the microwave method, the microwave should be set at around a 400 W or medium setting and the flask swirled every minute. The solution should be heated until all crystals are dissolved. This is best viewed against a light background. Crystals appear as translucent crystals. These will interfere with sample migration if not completely dissolved.
5. **The gel must be cooled to between 50˚C and 60˚C degrees before pouring.**

Gel pouring with casting dams

1. Fit the casting dams over each end of the tray and place onto a level surface. The dams should be fitted so that there is no gap between the sides of the tray and the groove in the dams. This will ensure that there is no possibility of gel leakage.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Gel pouring with model E3100

1. Fit the casting gates into the grooves in the unit which are 10 mm from the platinum wire. Ensure that these are seated as deeply as possible. This will ensure that a good seal is formed and that there is no possibility of gel leakage. A small amount of vaseline or sealing grease on the bottom and side edges of the gates will give further leakage protection.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Gel pouring using tape method

1. Autoclave or plastic backed general tape should be used. A length 5 cm longer than the width of each end of the tray should be cut. One length should be placed over one end of the tray folded and the edges sealed securely. Repeat for the other end and place onto a level surface for gel pouring.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Gel pouring using the flexicaster

1. Level the flexicaster base by adjusting the feet so that the bubble is exactly central.
2. Insert the desired length tray into the flexicaster such that one end of the tray is pushed up and seals against the silicone mat of the permanent end of the flexicaster.
3. Position the movable end of the flexicaster so that the silicone mat is pushed against the other end of the tray.
4. Turn the cam so that the silicone mat tightly seals against the side of the tray. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
5. Allow the agarose to set, ensuring that the gel remains undisturbed.
6. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Running the gel

1. Mix the sample to be loaded with sample buffer (see solutions for common sample buffers). Usually 3 µl of sample buffer is adequate but less may be used with sample volumes of less than 10 µl.
2. Fill the unit with buffer until the gel is just flooded with buffer. This will give the fastest resolution times. For enhanced quality of resolution of sample, fill the unit to 5 mm above the gel.
3. Load the samples into the wells using pipettes. Multi-channel pipettes can be used for loading samples with MultiPipette compatible combs, see listing in accessories for identification of these.
4. Carefully place the lid on the tank and connect to a power supply.
5. Typically gels are run at between 90 and 150 V. However, maximum voltage is indicated on the serial badge of each unit. It should be noted that a higher voltage generally give faster but poorer quality sample resolution.

Gel staining and viewing

All trays and the E3100 unit allow staining to be performed without removing the gel from the tray if this is preferred.

1. Transfer the gel to a vessel containing the appropriate volume of 0.5 µg/ml ethidium bromide stain for 15...30 minutes, see solutions for stock stain concentration and adjust to the volume used accordingly. The entire gel should be covered. (Caution: ethidium bromide is a suspected carcinogen and the necessary safety precautions should be undertaken).
2. De-stain the gel for 10–30 minutes in distilled water again ensuring the gel is completely immersed.
3. Rinse the gel twice for a couple of seconds with distilled water.
4. Transfer the gel to a UV Transilluminator.
5. The samples will often appear as brighter, clearer bands when photographed or viewed using a gel documentation system. However if the gel bands are too faint then the staining procedure should be adjusted so that there is less de-staining. If there is too much background then the staining procedure should be adjusted so that there is more de-staining.

1x TAE 40 mM tris (pH 7.6), 20 mM acetic acid, 1 mM EDTA

- 50 x (1l) dissolve in 750 ml distilled water:
- 242 g tris base (FW = 121)
- 57.1 ml glacial acetic acid
- 100 ml 0.5 M EDTA (pH 8.0).
- Fill to 1 litre with distilled water.

1x TBE 89 mM tris (pH 7.6), 89 mM boric acid, 2 mM EDTA

- 10 x (1l) dissolve in 750 ml distilled water:
- 108 g tris base (FW = 121)
- 55 g boric acid (FW = 61.8)
- 40 ml 0.5 M EDTA (pH 8.0)
- Fill to 1 litre with distilled water.

Sample Loading Dye

- 10x sample buffer stock consists of 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanole FF in 1x TAE buffer. Only 1–10 ml of the 10x loading dye should be prepared.

Ethidium Bromide Solution

- Add 10 mg of Ethidium Bromide to 1 ml distilled water.

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

E3100, E3200, E3300, E3400, E3500, E3600

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

Consort bvba

Parklaan 36, B-2300 Turnhout, Belgium
Tel: (+32) (0)14 41 12 79
Fax: (+32) (0)14 42 91 79
Sales: sales@consort.be

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.

Consort

www.consort.be

Parklaan 36 • B-2300 Turnhout • Belgium
Tel: (+32) (0)14 41 12 79
Fax: (+32) (0)14 42 91 79
Sales: sales@consort.be
Support: support@consort.be
Information: info@consort.be