

Electro-4 Gel Tank

USER INSTRUCTION MANUAL

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HB-E4GT-MAN



FS31999

ELECTRO-4 GEL TANK

Warranty

Thermo Electron Corporation guarantees that the Electro-4 Gel Tank you have received has been thoroughly tested and meets its published specification.

This warranty is valid for 12 months only if the product and functions have been used according to the user instruction manual.

This warranty period can be extended to a total of **24 months (free of charge)** by completing the warranty registration card supplied with the instrument, also available online at www.thermo.com/warrantylog.

No liability is accepted for loss or damage arising from the incorrect use of the Electro-4 Gel Tank. Thermo's liability is limited to the repair or replacement of the unit or refund of the purchase price at Thermo's option. Thermo is not liable for any consequential damages.

Thermo reserves the right to alter the specification of the Electro-4 Gel Tank without prior notice. This will enable us to implement developments as soon as they arise.

The Thermo Electro-4 Gel Tank is for research use only.

Read the Instruction Manual carefully before using the Electro-4 Gel Tank to ensure that you obtain the best results from the unit.

We are always interested to receive feedback on our products and services. Please e-mail your comments to quality@thermohybid.com or fill in our online customer satisfaction survey at: www.thermo.com/css.

ELECTRO-4 GEL TANK

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CHAPTER 1

ELECTRO-4 GEL TANK

Introduction

Submarine agarose gel electrophoresis is the standard method for size separation of nucleic acid fragments, both DNA and RNA.

The Thermo Electro-4 Gel Tank is a versatile high sample throughput, horizontal, agarose gel electrophoresis system. Up to four gels may be run simultaneously, stacked one on top of the other. Three different length gel moulds are available with the system to run mini, midi or maxi length gels for a range of applications from rapid plasmid screening to RFLP (Restriction Fragment Length Polymorphism) analysis.

The system is designed to co-ordinate with a 96 well format microtitre tray. Thus the gel combs are designed so that gels may be directly loaded using a 4 or 8-channel multi-pipette, thus reducing loading time dramatically.

The system is uniquely adapted for centrifugal blotting.

Thermo continues to offer full service and technical support for all its products.

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Alternatively, contact your local authorised distributor.

CHAPTER 2

ELECTRO-4 GEL TANK

Unpacking & Installation

Unpacking

Unpack the Electro-4 Gel Tank from its box and check that the unit and the components inside have not been damaged in transit and that the system is complete.

Check List

- 1 x Moulded Electro-4 Gel Tank with hinged lid, fitted with 2 electrodes - individually wrapped
- 4 x UV transparent Midi casting Trays (12cm x 9cm)
- 8 x End Dams including foam sponge
- 4 x Comb 1: 10:18 tooth
- 4 x Comb 2: Prep: 14 tooth
- 4 x Midi sponge
- 1 x H Section (300mm)
- 1 x Spirit Level Bubble
- 1 x Green Mat
- 1 x 8 Channel Helper
- 2 x Buffer Recirculation straws
- 1 x Red Power Lead
- 1 x Black Power Lead
- 1 x Instruction Manual

If any item is missing or damaged, please contact Thermo or Thermo's authorised distributor.

Installation

Remove the wrappings from around the electrode cassettes. Place the electrode cassette in the slot appropriate to the number of stacked gels you have selected to run. The electrode heights in the Electro-4 Gel Tank are adjustable to optimise the evenness of DNA/RNA migration in the four gels and to minimise buffer consumption when less than four gels are being used.

- For one gel place the electrode in the bottom slot.
- For two gels place the electrode in the bottom slot.
- For three gels place the electrode in the middle slot.
- For four gels place the electrode in the top slot.

The Electro-4 Gel Tank must be levelled before use. This ensures even band migration. Level the unit by placing the spirit level bubble supplied in the centre of the base of the tank and adjust the levelling feet until the unit is stable and level.

NB: If the Electro-4 Gel Tank is not used as specified in the manual, the protection provided by the equipment may be impaired.

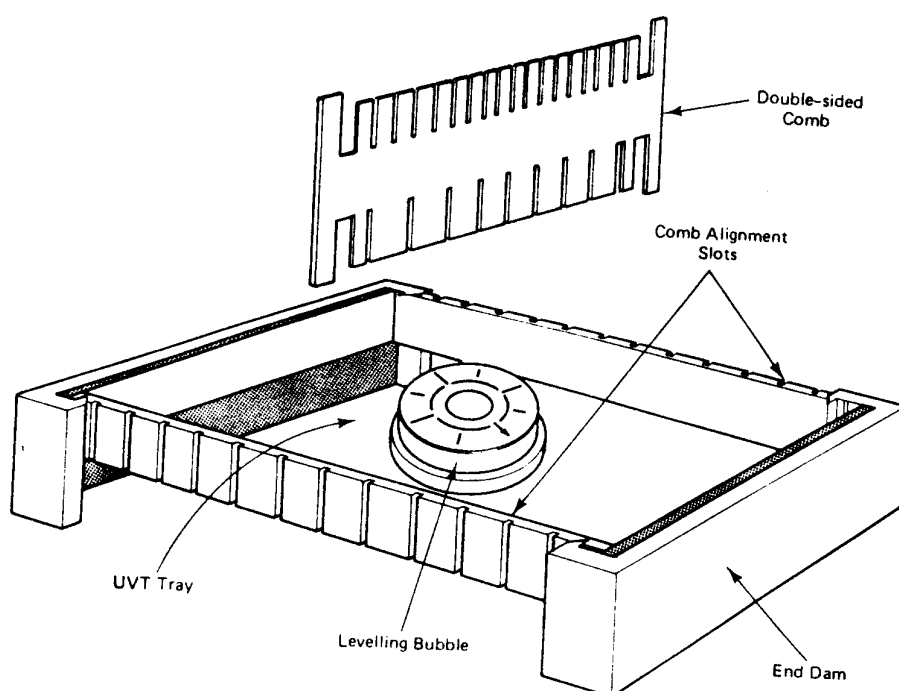
CHAPTER 3

ELECTRO-4 GEL TANK

Methodology

Gel Casting

Figure 3.1:



The ends of the gel moulds are sealed using the end dams provided. These should be clipped into place at the ends of the gel moulds such that the top edge of the end dam is flush with the top edge of the gel mould. Once snapped in place, the seal created between the neoprene rubber pads of the end dams and the gel tray creates a leak proof gel mould.

Level the mould by placing the spirit level bubble provided in the centre of the gel tray, then, by minor adjustment of the end dam, adjust the mould to be perfectly horizontal.

Two types of gel tray combs are provided; one comb has a preparative well former with two marker tracks on one face and 12 teeth on the other face plus two marker tracks. The other comb has ten teeth on one face (designed for eight tracks to be loaded with a multi-channel pipettor plus two marker tracks) and 18 tracks on the other face (16 tracks to be loaded with an eight channel multi-pipette and two marker tracks).

The combs for the Electro-4 Gel Tank slot positively into position in the gel trays, which ensures that the teeth are always vertical and at the correct depth for optimal well formation. The combs may be positioned at 1 cm intervals, enabling variable length runs or multiple length runs within one gel. Pour the molten agarose solution (cooled to approximately 60°C) into the gel tray mould. Slot the comb or combs into position and leave to set. When the agarose has set, the comb(s) and then the end dams should be carefully removed leaving the gel mould ready for use.

Sample Loading & Running

We strongly recommend using 0.5 x TBE as running buffer in the Electro-4 Gel Tank. TAE buffers are not recommended for this gel system and in no circumstances should it be run with this buffer at voltages greater than 50V (see *Table 3.1* for recommended running conditions).

TBE (Tris borate EDTA) buffer should be prepared as a concentrated stock solution of 5 x TBE:

5 x TBE 54.0g Tris
27.5g Boric Acid
20ml 0.5M EDTA (pH8)

A 1:10 dilution of the concentrated stock results in a solution of 0.045M Tris borate, 0.001M EDTA. The agarose gel itself should also be prepared using the 0.5 x TBE buffer. Select the correct agarose concentration for efficient size separation (see Appendix II).

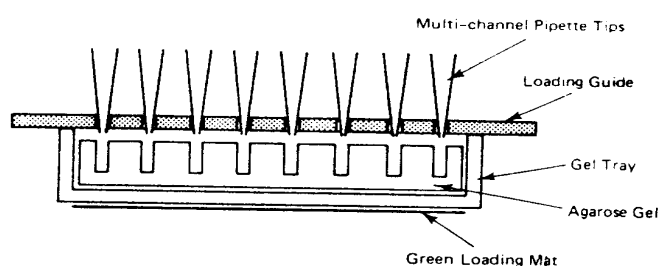
Table 3.1: Recommended Running Conditions

Buffer	0.5 x TBE (only)
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Voltage	0 - 100V
Gel	0.8 - 1.2% Agarose
DO NOT USE LOW MELTING POINT AGAROSE	
Mini Trays (6cm x 9cm)	
Midi Trays (12cm x 9cm)	
Maxi Trays (18cm x 9cm) (Re-circulate the buffer)	

Sample Loading

Figure 3.2: Cross section to show use of loading guide



Samples may be loaded under buffer, but to maximise the potential of the Electro-4 Gel Tank it is strongly recommended that samples are loaded on the bench. To semi-dry load simply pipette a small amount of buffer roughly over the sample wells, then load the samples into the wells using a multi-channel pipette. (See Appendix III for commonly used gel-loading buffers.)

To aid loading: -

1. A green loading mat has been supplied to give contrast. This can be used for both semi-dry and wet loading.

2. If a multi-channel pipette is used, we also recommend the use of the 8-channel helper supplied. This helps to both align the tips and provides support when loading the samples.

Once the samples are loaded simply submerge the gel under the buffer in the gel tank. Ease the sample-loaded end of the gel in first and gently lower the gel tray in to allow the buffer to completely flood the top surface of the gel.

Approximate buffer volumes required: -

1 gel	250ml
2 gels	500ml
3 gels	750ml
4 gels	1 litre

See Installation (Chapter 2) for correct positioning of electrodes.



When running only one gel, always ensure the buffer level is sufficient to flood the gel and cover the platinum wire visible on the electrode cassettes.

Gel Running

When the gel trays are in position close the lid and carefully connect the leads into the back of the unit and subsequently the power supply.

Switch on the power supply and run the gel under the conditions outlined in *Table 3.1*.

When the dye front has migrated the desired distance in the gel, switch off the power supply, disconnect the power leads from both the power supply and the Electro-4 Gel Tank itself and open the lid.

Remove the gel trays from the Electro-4 Gel Tank and immerse the gel in a solution of running buffer containing 0.5µg/ml of ethidium bromide for approximately 20 minutes to stain the gel. A short destaining step (5 minutes) in running buffer will remove excess stain and help to reduce background fluorescence.

Alternatively, many researchers run the gel in buffer containing ethidium bromide. This allows the DNA to be visualised directly at any time during the run.

**ETHIDIUM BROMIDE IS A POTENT MUTAGEN. GLOVES SHOULD BE WORN
 WHEN HANDLING SOLUTIONS CONTAINING THIS DYE.**

The ethidium bromide stained DNA is visualised by fluorescence under ultra violet light. The gel trays supplied with the Electro-4 Gel Tank are UV transparent, therefore the gel need not be removed from the mould to be visualised and photographed.

It should be noted that short wave UV light (<280nm) may cause damage to DNA molecules, therefore long wave light sources (300-360nm) should be used to visualise DNA where recovery and subsequent manipulation steps are to be carried out.

Buffer Recirculation

If running this system with TAE buffers or using the maxi trays, buffer recirculation is recommended. Connect thin bore laboratory tubing to the ends of the buffer recirculation straws provided and hook these over either end of the Electro-4 Gel Tank locating the external down pipe firmly in the notch. Close the lid and circulate the buffer via an external peristaltic pump.

CHAPTER 4

ELECTRO-4 GEL TANK

Nucleic Acid Transfer

The Thermo Electro-4 Gel Tank has been specifically designed to facilitate nucleic acid blotting protocols. Conventional blotting protocols, e.g. vacuum blotting using the Thermo Vacu-Aid, may be readily performed, but two blotting protocols have been developed and optimised specifically for the Electro-4 Gel Tank: sandwich blotting and ultra fast centrifugal blotting.

Sandwich Blotting

(See *Figure 4.1* and *4.2.*)

After electrophoretic resolution, remove the gel from the casting tray. Treat in a sandwich box as follows: -

1. **Depurination**

Depurination to break the DNA fragments into smaller fragments suitable for transfer is recommended for all DNA fragments larger than 10kb and may assist transfer of smaller fragments. Add 0.25M Hcl to completely cover the gel and soak for ten minutes at room temperature. Care should be taken not to over depurinate the DNA, as very small DNA fragments may not bind efficiently to some membranes.

Figure 4.1:

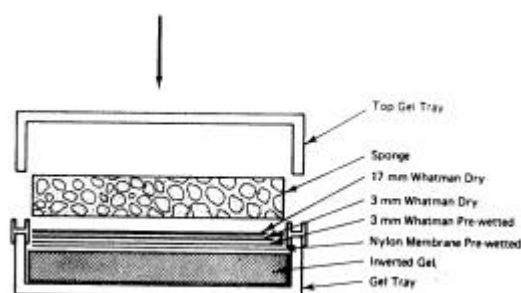
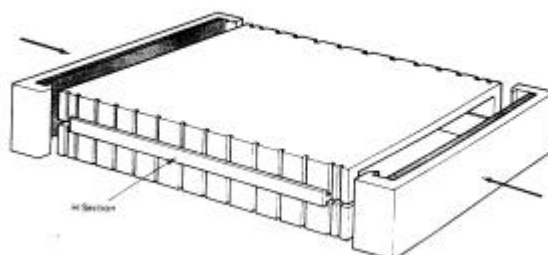


Figure 4.2:



It should also be noted that exposure to UV light will also break up the DNA into small fragments.

2. Replace the depurination solution with denaturing solution (1.5M NaCl, 0.5M NaOH) and soak for 15 minutes.
3. Replace the denaturing solution with neutralising solution (1.5M NaCl, 0.5N Tris Cl pH7.2, 1mM Na₂ EDTA).

At the end of the pre-treatment stages drain off the neutralisation buffer and replace the gel, inverted, in the casting tray and attach the two end dams so that each is flush with the bottom of the gel mould. Then proceed as follows: -

4. Overlay the gel with a pre-cut, pre-wetted sheet of nylon membrane.
5. Overlay with pre-cut filter papers as follows:
 - 1 pre-wetted 3MM Whatman™ paper
 - 1 dry 3MM Whatman™ paper
 - 1 dry 17MM Whatman™ paper.
6. Overlay with the sponge provided, which should be softened with water and then squeezed dry before use.
7. Cut two suitable lengths of the silicone 'H' section and locate these in position on the sidewalls of the gel tray.

Overlay this assembly with a second gel tray squeezing it into place on the first gel tray to compress the sponge and locate in the 'H' section. Seal the ends with the end dams to form a blotting cassette.

8. This effectively promotes capillary transfer whilst minimising the extent of gel collapse, which results from conventional capillary blotting methods utilising an unrestricted weight on a stack of paper towels. Complete transfer typically occurs in approximately 4-6 hours, with sensitivity equal to that of conventional capillary blotting performed overnight.
9. Dismantle the blotting cassette, discard the gel and filters and rinse the sponge ready for re-use. Process the membrane to fix DNA, either by UV cross-linking, or by baking.
10. Hybridise to the labelled probe in hybridisation bottles in a Thermo Hybridisation Oven. This is the simplest method of hybridisation and produces excellent, reproducible results with a high level of safety and convenience.

The rotisserie action of the Ovens enables hybridisations to be performed in minimal probe volumes with the fluid moving continually over the membrane.

Ultra-Fast Centrifugal Blotting

This technique is adapted from the method of Wilkins & Snell (see Appendix IV). Gel processing by depurination, denaturation and neutralisation is carried out exactly as for capillary transfer. Assemble the 'gel-membrane-sponge' sandwich as for capillary transfer, then place in a centrifuge with a rotor designed to hold microtitre plates.

NB: The gel tray sandwich completes with end dams fits Wifuge and MSE centrifuges. Compatibility with other centrifuge rotors, e.g. Hermle, can be achieved by discarding the end dams in favour of tape. Due to width restrictions of the rotor, the gel trays do not fit Heraeus, or Sorvall™ centrifuges.

Centrifuge for 20 minutes at 40g (typically 400-600rpm), such that the centrifugal force drives the DNA out of the gel and on to the membrane. Efficient DNA transfer is typically achieved in 20 minutes. This procedure is suitable for gel concentrations from 0.75% to 3%.

Disassemble the gel trays, discard the gel and Whatman™ filters and rinse the sponge for re-use. Fix the DNA to the membrane and hybridise as above in a Thermo Hybridisation Oven.

CHAPTER 5
ELECTRO-4 GEL TANK
Technical Specifications & Ordering Information

Technical Specifications

Catalogue N° :	HB-E4-GT
Dimensions:	140mmW x 115mmH x 290mmL
Weight:	1.3kg
Maximum gel thickness:	10mm
Working buffer volume:	300ml per gel
Standard Accessories:	4 midi-casting trays (120 x 90mm)
.....	8 gel tray end dams
.....	4 x 18 & 8 sample gel combs
.....	8 channel helper
.....	Shrouded power leads
.....	H spacer
.....	4 blotting sponges
.....	Levelling bubble
.....	Loading mat

Ordering Information

Mini Gel Casting Tray (60 x 90mm).....	HB-E4-CTN
Midi Gel Casting Tray (120 x 90mm)	HB-E4-CTD
Maxi Gel Casting Tray (180 x 90mm).....	HB-E4-CTX
Gel Tray End Dams (set of 4).....	HB-E4-ED4
10:18 Tooth Comb (multi-pipette compatible)	HB-E4-C1
Prep: 14 Tooth Comb	HB-E4-C2
H Section for Blotting	HB-E4-HS

Replacement Right Electrode	HB-E4-MELECR
Replacement Left Electrode.....	HB-E4-MELECL
Power Cords.....	HB-MG-PSUL
8 Channel Helper.....	HB-E4-CH
Blotting Sponges (pack of 10)	
Mini	HB-E4-SP
Midi.....	HB-E4-SM
Maxi	HB-E4-SX
Level Bubble	HB-E4-LB
Loading Mat.....	HB-E4-LM

APPENDIX I
ELECTRO-4 GEL TANK
Cleaning & Maintenance

For optimal results, the Electro-4 Gel Tank should be kept clean. As with all acrylic lab ware the unit should not be cleaned with ethanol or any other organic solvent. Use warm water and a mild detergent to remove any debris and residue from the unit.

APPENDIX II
ELECTRO-4 GEL TANK
Recommended (% W/V) Agarose for
Efficient Size Separation of Linear DNA Molecules

Amount of Agarose in Gel (% W/V)	Efficient Range of Size Separation (kb)
0.3	5 - 60
0.6	1 - 20
0.7	0.8 - 10
0.9	0.5 - 7
1.2	0.4 - 6
1.5	0.2 - 3
2.0	0.1 - 2

APPENDIX III
ELECTRO-4 GEL TANK
Commonly used Gel Loading Buffers

Type of Loading Buffer	Constituents
I	6 x buffer: 0.25% bromophenol blue 0.25% xylene cyanol 40% (w/v) sucrose in H ₂ O. Store at 4°C
II	10 x buffer: 0.25% bromophenol blue 0.25% xylene cyanol 25% Ficoll (type 400) in H ₂ O Store at room temperature
III	6 x buffer: 0.25% bromophenol blue 0.25% xylene cyanol 30% glycerol in H ₂ O Store at 4°C
IV	6 x buffer: 0.25% bromophenol blue 40% (w/v) sucrose in H ₂ O Store at 4°C

APPENDIX IV
ELECTRO-4 GEL TANK
References

Maniatis, T., Fritsch, E.F. & Sambrook, J. 1982

Molecular cloning: A Laboratory Manual,
New York: Cold Spring Harbour, 2nd Edition

Wilkins, R.J. & Snell, R.G. 1987

Centrifugal transfer and sandwich hybridisation permit 12-hour Southern blot analysis.
Nucleic Acids Research, 17: 7200

