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**INSTRUCTION MANUAL
OF
HORIZONTAL GEL ELECTROPHORESIS
APPARATUS**

LIGOGEL SYSTEM

(CAT No. THLGR-000308)
(OLD CAT. No. 65-S8000)

Ligogel – *For DNA and protein separation*

TechResource
Scientific Pvt Ltd

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Dear Customer,

Congratulations on procuring the Ligogel system,

LigoGel is a comb and running tray combo for quick electrophoresis of bio-molecules in agarose. The compact size requires less agarose but provides sufficient length for good band resolution. Samples can be run quickly; be it genomic or plasmid DNA, restriction-digested DNA, PCR products, RNA or large proteins.

The tray is designed such that the agarose forms a flattened convex shape on the tray without spilling over the edges. This eliminates the need for costly casting-stands or cumbersome tape.

Additionally, the tray base is UV-transparent and hence can be placed straight onto a UV trans-illuminator or in a gel doc. It also has a UV-fluorescent ruler in the millimeter scale printed on the back for running distance comparison.

Any horizontal gel electrophoresis apparatus can be used for providing the electrical conductivity.

The Ligogel system helps to run a Ligogel quickly, using the least amount of buffer hence preventing buffer wastage.

TechnoSource products are manufactured and marketed by TechResource

TechResource 188, Nagdevi Street, 2nd floor, Mumbai 400 003.

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Essential components of the system

1. Ligogel tank, fitted with 4 mm male connector and 0.25 mm thick electrodes, 1 pc
2. UV-transparent slide with gel gripping design, 2.3 (w) x 8.5 (l) cm, 1 pc
3. Comb, PMMA, 1.5 mm, 4 well, 1 pc
4. Lid for Ligogel
5. Gel slide lifter
6. Electrical cords with male and female connectors
7. Instruction manual sticker

Caution

Use the apparatus with care. It should be washed and rinsed with soft water, air dried at room temperature and stored covered.

It should not be

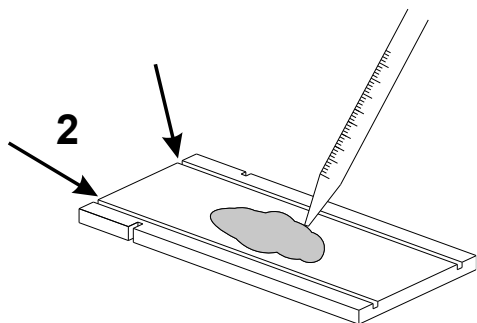
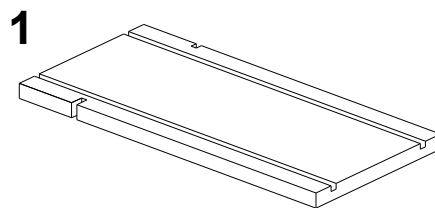
- Subjected to impact
- Exposed continuously to temperatures outside the range of 0-40°C.
- Handled with abrasive or sharp objects,
- Exposed to organic solvents such as aromatic or halogenated hydrocarbons, ketones, esters, alcohols over 30% or concentrated acids over 25%,
- Exposed to other solutions which will attack plastics (acrylic, nylon and polycarbonate)

Other items needed to perform horizontal gel electrophoresis

- | | |
|--|--|
| 1. A constant current power supply with minimum rating of 150 V, 50 mA | b. Agarose powder |
| 2. To clean the apparatus | c. Pipettes (10 ml and 5 ml) |
| a. Abrasive and enzyme free detergent | d. Ethidium bromide (EtBr) [optional] |
| b. De-mineralized tap water | e. Micropipettes |
| c. Squirt bottle containing distilled water | f. Leveling table [optional] |
| 3. To assemble the apparatus | g. Magnetic stirrer with hot plate/ microwave oven |
| a. Tissue paper | 5. To prepare and load the samples |
| b. Blotting paper | a. Polypropylene tubes, |
| 4. To prepare the agarose gels | b. Dye (bromophenol blue) in buffer |
| a. Stock buffer | c. High speed centrifuge |
| | d. Micropipettes |

Using LigoGel

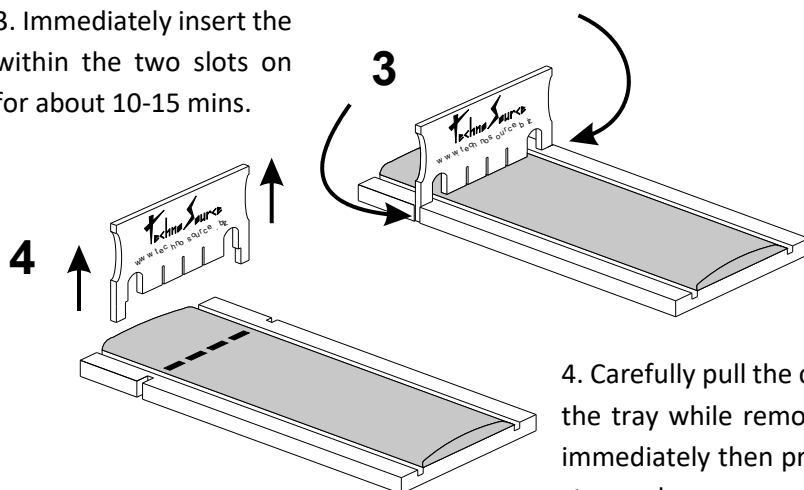
1. Clean and dry the comb and the base tray. Place the tray on a levelled surface with the groove side facing up. (ensure that the grooves are clean and free of residual agarose)



2. Prepare 10-15 mL of the required percentage of agarose (add EtBr if required). Cool the agarose to about 50° C. Carefully pour the agarose solution onto the tray between the parallel grooves using a 10 mL pipette (or you can directly pour it from a beaker).

(The agarose should not flow over the grooves for this will result in it flowing off the tray. Pouring agarose solutions below 1% requires skill which comes with practice)

3. Immediately insert the comb within the two slots on for about 10-15 mins.



comb such that the legs of the comb sit the tray. Allow the agarose to solidify

4. Carefully pull the comb out vertically. Use one hand to hold the tray while removing the comb. If you want to run a gel immediately then proceed to step 5, else store in the LigoGel storage box.

5. Place the tray on the gel slide lifter and place it on the platform of the LigoGel horizontal electrophoresis tank

6. Pour buffer into the tank till the gel is just submerged and load the samples with a micropipette.

(The wells will accommodate 7-20 μ L depending on the volume of agarose poured and the skill of loading.)

7. Connect the cords to a power supply and run at 80 - 200 V.

8. When the gel has run, place the tray directly on a UV-transilluminator / gel doc.

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Tips and notes

1. Preparing agarose: In a clean, dry conical flask add agarose powder (low electro-osmosis grade) followed by appropriate amount of stock buffer, and D/W. Introduce a Teflon coated magnetic spin bar into the flask, cover its mouth with foil and place it on a hot plate magnetic stirrer. While it is being heated stir at medium speed (avoid bubble formation). The solution should turn clear after which the magnet is removed, the solution is allowed to cool to the appropriate temperature (see notes in 6 below) and poured into the gel tray.
2. EtBr should be added only to the agarose solution which can be cooled to 50-55 °C.
3. Storage of gelled agarose: Store the gelled agarose in air tight containers at 4 °C in required aliquots. In case the agarose is repeatedly melted, its concentration will increase.
4. Reuse of cast gel: If the gel needs to be reused, connect the apparatus to a power supply (designed for safe overnight experiments) and run the gel overnight at a constant current of 10mA to migrate the DNA from the gel. Check that the gel is free of any DNA before re-using.
5. The migration of DNA in an electric field depends on a number of factors such as conformation (super-coiled, nicked or linear), composition of buffer (type, ionic strength, pH), presence of EtBr and concentration of agarose. In an electric field the DNA first orients itself in an end on position and then migrates at rates that are inversely proportional to the $\log_{10}$ number of base pairs. Larger molecules migrate slower than expected because of greater frictional drag and because they worm their way through the pores less efficiently than small molecules.
6. Guide in choosing gel concentrations and voltage for separating DNA, approximate running positions and special comments.

Agarose concentration in gel (% w/v)	Range of separation of linear DNA molecules (kb)	V gradient V/ cm	Approximate running positions of		Notes
			Bromophenol Blue	Xylene cyanol	
0.3	5-60	5	3 kb	25 kb	Very fragile and difficult to handle. Opt for polyacrylamide gels if possible
0.6	1-20	5	1.7 kb	11 kb	
0.8	0.8-10	5	1 kb	0.7 kb	
1.0	0.5-6	10	0.5 kb	6 kb	
1.2	0.4-6	10	0.4 kb	3.5 kb	
1.5	0.2-3	15	0.3 kb	2.5 kb	
2.0	0.1-2	20	0.15 kb	1.3 kb	Pour agarose at 55°C in pre-warmed tray
3.0	0.1-1	20	0.1 kb	1.2 kb	