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**INSTRUCTION MANUAL
OF
HORIZONTAL GEL ELECTROPHORESIS APPARATUS

TUBBYSUB
(E2300151/ E2300152/ E2300153/ E2300154)**

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Components of the Ready-to-use system (TubbySub):

1. Tank with removable electrodes & male connectors
2. UV-opaque/ UV-transparent tray for gel size
 - 15 (w) x 10 (l) cm (TubbySub)
3. Two PMMA combs (1.5 mm thick)
 - 15 well (TubbySub)
4. Lid
5. Electrical cords with male & female connectors (1 pair)

Additional handy components available on request:

1. Gel-casting stand
2. Gel slicer

CAUTION

Use the apparatus with care. Wash as directed.
Dry the connecting pins before attaching the electrical cords.

The apparatus 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 (aromatic or halogenated hydrocarbons, ketones, esters, alcohols over 30% or concentrated acids over 25%)
- ...exposed to other solutions which will attack plastic.

Other items needed to prepare & run a gel**To clean the apparatus:**

- Liquid detergent
- Brush with flattened bristles
- D/W in a squirt bottle

To prepare the agarose gel:

- Stock buffer
- Low EEO agarose powder
- 5 mL, 10 mL pipettes
- Ethidium bromide stock solution (EtBr)
- 200 µL & 20 µL micropipettes & their corresponding tips
- Magnetic stirrer
- Hot-plate/microwave
- Distilled water (D/W)
- Levelling table
- Flask

To assemble the set-up:

- Tissue/blotting paper
- Gummed tape (1/2 inch cloth/ electrical insulation tape)
- A pair of scissors

To prepare & load the samples:

- Polypropylene tubes,
- Tracking dye (bromophenol blue) in appropriate buffer,
- Tube rack (immersible in boiling water)

Other equipment:

- A power supply
- High-speed centrifuge with rotor for polypropylene tubes
- Levelling table (optional)
- Trasilluminator/Gel documentation system

PREPARING STOCK SOLUTIONS**EDTA (0.5 M, pH 8.0)**

In 80 mL D/W add 18.61 g disodium EDTA.2H₂O. Stir vigorously. Adjust pH to 8.0 with NaOH (approx 2.0 g). The solution will remain turbid till the pH remains below 8.0.

Ethidium Bromide (10 mg/mL)

Add 10 mg of ethidium bromide to 10 mL of D/W. Stir on a magnetic stirrer for several hours to ensure the dye has dissolved. Store at room temperature in a dark bottle.

Tank Buffer

50X TAE stock solution: To 700 mL D/W add 242 g Tris base, 57.1 mL glacial acetic acid and 100 mL of 0.5 M EDTA (pH 8.0). Dissolve and make the volume to 1 L with D/W.

1 X working solution: Take 20 mL of stock TAE solution and make up the volume to 1 L with D/W.

5X TBE stock solution: To 800 mL of D/W add 54 g of Tris base, 27.5 g boric acid and 20 mL of 0.5 M EDTA (pH 8.0). Dissolve and make the volume to 1 L with D/W.

1 X working solution: Take 200 mL of stock TBE solution and make up the volume to 1 L with D/W.

6X Gel loading buffer

To 50 mL D/W add 0.25 g bromophenol blue, 0.25 g xylene cyanol (optional). Stir to dissolve. Add 40 g sucrose. Dissolve and make up the volume to 100 mL with D/W. Store at 4°C for short durations and at -20°C for longer durations.

The choice between TAE and TBE can be made based on the following properties

...TAE has low buffering capacity and will get exhausted if electrophoresis is carried out for prolonged times.

...TBE is more expensive than TAE.

...Double stranded DNA fragments migrate faster through TAE than through TBE.

...The resolving power of TAE is better for higher molecular weight and for supercoiled DNA. TBE is better for lower-molecular weight DNA.

Choice of Agarose concentrations

Agarose concentration in gel (% w/v)	Range of separation of linear DNA molecules (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

Use the table below to obtain the amount of **Agarose (in g)** to weigh for the required percentage of gel and gel volume. Melt agarose in 1x TAE/ TBE buffer.

Tray size (cm)	No. of wells per comb	Volume of gel (mL)	Agarose percentage				
			0.8%	1%	1.2%	1.5%	2%
3 (w) x 10 (l)	4	20	0.16 g	0.20 g	0.24 g	0.30 g	0.40 g
7 (w) x 10 (l)	8	40	0.32 g	0.40 g	0.48 g	0.60 g	0.80 g
15 (w) x 10 (l)	15	100	0.80 g	1.00 g	1.20 g	1.50 g	2.00 g

The approximate volume of sample that each well will accommodate is about 30 μ L.

ASSEMBLING THE APPARATUS (summary)

1. Clean and dry the apparatus.
2. Seal the sides of the tray with the tape (insulation/ sticking plaster) or clamp tray in the casting stand
3. Slide comb in the slot of tray
4. Prepare the required percentage of agarose. Add the EtBr (optional).
5. Place tray on a levelled surface and pour the agarose solution (at about 50°C) into the tray
6. Allow to cool for about one hour, remove the tape
7. Place the tray in the tank
8. Pour buffer into the tank such that the gel is just submerged
9. Remove the comb gently
10. Load the samples with a micropipette
11. Cover the tank, connect to a power supply and carry out electrophoresis at 80-140 V.
12. After the separation is done, turn off the power supply, disassemble the apparatus & treat the gels appropriately for staining / blotting / autoradiography.

DETAILED PROCEDURE FOR ASSEMBLING THE APPARATUS.**I Cleaning the Apparatus:**

1. As residual ethidium bromide may be present, it is advisable to use gloves while cleaning.
2. Rinse the apparatus thoroughly with water. In case the apparatus is dirty, clean it carefully with a mild detergent & a brush.
3. Rinse with tap water, then with D/W. Invert to dry.
4. Exert utmost care while cleaning, rinsing & drying the UV-transparent tray to avoid scratching the UV-transparent surface. Stand the tray on its edge to dry
(The water should drain off evenly from the surface of the tray, else, clean again)

II Sealing the tray:

1. To prepare the UV-transparent tray for gel casting, cut two 5 / 8/ 16 cm strips of tape. Tape both the ends of the tray making sure that the tape is high enough (1/2" wide gummed cloth or electrical insulation tape is well suited) to contain the amount of agarose solution poured. Place the tray on an abrasive free, levelled surface. **Do not use brown masking or cello tape** as these damage the UV-transparent surface.
Ensure that:
 - a) the tape does not protrude below the bottom of the tray else agarose will set at a slant; &
 - b) there are no crinkles along the tape, through which molten agarose could leak.
2. Else, use the gel-casting stand (available as an accessory) to set the agarose in the tray. To do so, turn the screw anti-clockwise & push the clamp back. Place the tray in the stand & turn the screw clockwise to seal the edges of the tray. **Do not** over-tighten.

III Casting the gel:

1. Slide the comb in the slot of UV-transparent tray. Comb will get locked in the right position.
2. Ensure that the agarose solution is at 50°C & add EtBr solution (final concentration 0.5 µg/mL) if desired. Pour the hot solution into the tray (about 4-5 mm deep). Allow the gel to set for 60 min. Longer when ambient temperature is higher.
3. Pour some buffer over the comb & carefully lift the comb. Wash the comb. Drain the buffer into the electrophoresis tank. Peel the tape off from the ends. Place tray on the platform such that the wells are closer to the (black) cathode. The wells may be cleaned by gently flushing them with buffer.

IV Loading the samples & performing the electrophoresis

1. Pour the buffer into the cathode & anode chambers. Adjust the buffer level such that it is 1-2 mm above the gel surface
2. Place the black paper under the platform. The paper will provide a dark background, which makes it easy to locate the wells.
3. Load the samples containing glycerol & appropriate tracking dyes in the wells.
4. Cover the tank with the lid & connect the electrical cords (red to red & black to black) & run the gel at constant current or constant voltage of 70-80 V.
5. When the dye reaches the end of the gel, electrophoresis is complete.

Note in case you want the electrophoresis for a longer time; continue the run even after the dye front has moved out of the gel. For a shorter run, discontinue electrophoresis after the dye front reaches half way

V Dismantling the apparatus

1. After the run is complete, switch off the power supply, disconnect the electrical cords & remove the lid
2. With gloved hands lift the UV-transparent tray out of the tank, carefully drain off any buffer, wipe off adhering buffer from the underside of the tray & place it on a UV-transilluminator.
3. Ethidium bromide is a potent carcinogen therefore it has to be handled with care. The buffer & gel containing EtBr has to be disposed off as hazardous waste. DO NOT throw EtBr containing gels or solutions in the sink. DO NOT put bleach as it produces mutagenic products. The safest way to discard EtBr gels is to incinerate them. EtBr solutions should be treated with activated charcoal & the solution then discarded in the drain. The charcoal should be incinerated.
4. Store the electrophoresis tank buffer in a container; it can be reused for 2-3 times.
(Note the buffer may contain EtBr if the gel had EtBr incorporated in it).
5. Wash & dry all the parts. Place the entire dry unit in the storage box.