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ecosep@vsnl.net

**INSTRUCTION MANUAL
OF
VERTICAL GEL ELECTROPHORESIS APPARATUS**

TUBBYPAGE

(E0300142/E0300143)

TechResource

188, Nagdevi Street, 2nd Floor, Mumbai 400003.

Tel: 022 28937286

Email: techresource@vsnl.net

Components of the Ready-to-use system, TubbyPage:

1. Lower anodic tank with electrode & male connector
2. Cathodic electrode unit (CEU) for *in situ* gel casting fitted with upper tank, male connector & electrode
3. Resilient silicone gasket set (one short, one long) for CEU
4. Two notched & two rectangular glass plates
5. Four 1.5 mm thick PMMA spacers
6. Two PMMA combs (1.5 mm thick, 13 well)
7. Set of binder clips
8. Electrical cords with male & female connectors (1 pair)

Additional handy components available on request:

1. Gel-casting stand
2. Glass plate stand
3. Gel sandwich opener
4. Agarose dispenser

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 polyacrylamide gels:

- Acrylamide stock soln.
- Separating gel buffer stock soln.
- Stacking gel buffer stock soln.
- TEMED/ UVA or fluorescent light
- 10% freshly prepared ammonium persulphate/riboflavin
- SDS/ chaotropic agents
- Distilled water (D/W)
- 5 mL pipettes
- 200 µL & 20 µL micropipettes & their corresponding tips
- Flask (Stoppered side-arm flask & vacuum pump is optional).

To assemble the set-up:

- Tissue/blotting paper
- Petroleum jelly
- 5-10 mL pipette
- Glass-marking pen
- Agar (2%) or agarose (1-1.5%) in water
(an indigenous brand of agar/agarose is recommended).

To prepare the samples:

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

Other equipment:

- A power supply
- High-speed centrifuge with rotor for polypropylene tubes
- Levelling table (optional).

PREPARING STOCK SOLUTIONSAgar/ Agarose

In 100 mL D/W add either 2 g Agar or 1.5 g Agarose. Melt by heating in a microwave or on a burner.

Acrylamide Stock Solution (30%T, 2.7% C)

In 65 mL warm D/W dissolve 800 mg of Bis-acrylamide. Add 29.2 g Acrylamide. Dissolve on a magnetic stirrer. Make up the volume to 100 mL with D/W.

Acrylamide and bisacrylamide are neurotoxins whose effects are cumulative. Exercise due caution while preparing and handling the solutions containing these substances. Store at 4°C in dark bottle.

4 x Separating gel buffer (1.5 M Tris-Cl; pH 8.8)

In 30 mL D/W dissolve 9.08 g Tris. Add 3 N HCl till pH is 8.8. Make up the volume to 100 mL with D/W.

4 x Stacking gel buffer (0.5 M Tris-Cl; pH 6.8)

In 35 mL D/W dissolve 3.0 g Tris. Add 3 N HCl till pH is 6.8. Make up the volume to 100 mL with D/W.

10% SDS solution

In 80 mL warm D/W dissolve 10 g SDS. DO NOT autoclave.

10% Ammonium persulfate (prepare fresh)

In 1 mL D/W dissolve 100 mg APS.

Check that effervescence is produced on adding water; else, use a new batch of APS. Cover with foil. Store at 4°C.

1 x Tank buffer (25 mM Tris, 192 mM glycine, pH 8.3)

In 970 mL D/W dissolve, 3.0 g Tris, 14.4 g glycine. Add 10 mL 10% SDS solution. Make up the volume to 1000 mL with D/W.

Tracking dye stock solution

In 10 mL methanol dissolve, 50 mg bromophenol blue. Make up the volume to 100 mL with D/W.

2 x Loading buffer

Add 2.5mL of 4 x stacking buffer, 4.0 mL Glyderol, 0.5 mL tracking dye stock solution and mix. Add 2.0 mL 10% SDS solution and 1.0 mL 2-mercaptoethanol (optional).

Coomassie blue R-250 staining solution

In 400 mL methanol dissolve 1.0 g Coomassie blue R-250. Add 100 mL glacial acetic acid and 500 mL D/W. Filter through Whatman #1 filter paper.

Destaining solution

Add 400 mL methanol and 100 mL glacial acetic acid to 500 mL D/W.

Note: A convenient, cheap and fast way of de-staining gels is by boiling them in tap water in a microwave. Change the water (2-3 times) when it becomes blue.

Separating gel preparation chart

(values in mL unless specified)

	for 35 mL			
Gel %	8%	10%	12%	15%
Reagent				
D/W	16.1	13.3	11.2	7.7
30% Acryl. stock	9.1	11.9	14	17.5
4 x Sep. gel buff.	9.1	9.1	9.1	9.1
10% SDS (μL)	350	350	350	350
TEMED (μL)	21	14	14	14
10% APS (μL)	350	350	350	350

5% Stacking gel preparation chart

(values in mL unless specified)

Reagent	For 10 mL
D/W	7
30% Acryl. stock	1.65
4 x Stack. Gel buff.	1.25
20% SDS	0.1
10% APS	0.1
TEMED	10μL

ASSEMBLING THE APPARATUS

- Slide the CEU into the tank; fasten in place by tightening the screw on the back side.
- Put petroleum jelly on both the broad surfaces of each spacer.
- On the CEU, arrange the sandwich (consists of the notched plate, spacers, & rectangular plate).
- Hold the cassette in place by using the binder clips.
- Seal the bottom of the sandwich with 10-15 mL of molten agar/ agarose.
- Pour the acrylamide mixture(s) in between the plates of the sandwich & insert the comb.
- After the gel is set, remove the comb, fill the tanks with buffer, load the samples in the wells & apply electrovoltaic potential across the electrodes.
- 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:

- Wash the glass plates with mild liquid detergent using a brush.
 - Rinse with tap water, then with D/W & place them in the glass plate stand to dry.
- (The water should drain off evenly from the surface, else, clean again as clean plates ensure that the gel adheres to the plates)

II Assembling the sandwich:

1. Place the tank with the anode pin (red) facing oneself.
2. Loosen the screw on the CEU. With the gasket side facing oneself, place the CEU in the tank such that the back wall of the tank is between the main part of the CEU & the extending clasp. Tighten the screw(s).
3. Place the notched plate against the gasket (notched side up)
4. Spread a thin layer of petroleum jelly on the broad surfaces of each spacer & place them vertically along the edges of the notched plate with the bottom edges on the tank platform.
5. Place the rectangular glass plate against the spacers. Align the entire sandwich with the CEU.
6. Hold the sandwich in place using the binder clips; positioning them such that the pressure is over the gasket.
7. Ensure that the spacers are properly positioned & that the comb can be easily introduced into the sandwich & rest on the spacers. (If not, remove the binder clips & make necessary adjustments.)
8. Using a glass-marking pen, mark (i) the level to which the separating gel mixture has to be poured (ii) dots/dashes to indicate the centre/ bottom of each well.

III Sealing the sandwich:

1. Place the apparatus on a levelled surface.
(A levelling table can be used which is available as an accessory).
2. Melt the agar/agarose.
(If required, check the agar/ agarose to see if it sets firmly)
3. Transfer with a pipette or an agarose dispenser, 10-15 mL molten agar/agarose along the base of the sandwich, from one end to the other, such that it fills the gap between the plates. Allow it to cool for 10-15 min. so that it solidifies.

IV Casting polyacrylamide slab gels:

1. Mix double distilled or reverse osmosis purified water, stock acrylamide & buffer solutions in a flask (optional step - the mixture can be degassed in a side arm flask). Add catalyst, & a detergent, (if desired) & mix. Add the initiator, mix & pour the mixture upto the mark on the glass plate (or fill to the top & introduce the comb if the stacking gel is not used). Keep the remaining mixture aside.
2. Carefully overlay the acrylamide mixture with 0.5-1 mL of D/W with 1 mL glass pipette.
3. After 30 min, check if the mixture in the flask has polymerized. If so, check the polymerization of contents in the sandwich by tilting the apparatus sideways. Invert the apparatus to drain out the overlay solution.
4. Wash the top of the gel with D/W, invert the apparatus to drain & blot out last drop of water.
5. Repeat step 1 of section IV to polymerize the stacking gel. Pour the mixture till the brim of notched glass plate and insert the comb.
6. After 15 min, check if the stacking gel in the flask has polymerized. Pour D/W over the comb (or fill the cathodic chamber with DW) & pull it out slowly. Wash the wells with DW using pipette or syringe.

VI Loading & separating the samples:

1. Fill the cathodic chamber with tank buffer till the brim of rectangular plate.
2. Add buffer to the anodic tank such that it is above the level of the electrode.
Note: The ammonium persulphate used as the initiator of polymerization is a strong oxidising agent. Its concentration in the gel is approximately 2-5 mM. Residual persulphate in the stacking gel will leach into the wells & damage (oxidatively) the proteins. This damage can be minimized by
 - i) Using riboflavin as the initiator. (Load the samples & perform the electrophoresis in incandescent/subdued light)
 - ii) Using reducing agents such as 2-mercaptoethanol or dithiothreitol at high concentrations in the sample buffer
 - iii) Pre-running. Electrophoretically remove the persulphate before loading the sample. For this, load a small amount of 1x sample buffer to one of the wells, & migrate the dye in a voltage gradient till it reaches midway into the stacking gel. The electrophoresis is stopped & the samples are loaded.
3. Carefully introduce the samples into the wells. For best results, ensure that all the wells are filled to the same level, & that the ionic strength of all the samples is the same. To the unused wells, add 1x loading buffer.
4. Blot out any liquid that may be around the male connectors on the unit (CEU) to prevent their electrolytic corrosion.
5. Connect the electrical cords emanating from the power supply according to the colour codes (e.g. red to red). Operate under conditions of constant current. (1.5 mm gels Slimpage 10 mA; Ecopage 18 mA; Tubbypage 40mA) or at 110 V. For better resolution and less heat generation run at 80 V during stacking, 110 V during first half of separation and 140 V thereafter.

VII Dismantling the sandwich:

1. When the dye-front is about 5 mm from the bottom, switch off the power supply, & disconnect the electrical cords.
2. Store the buffers for reuse if the pH has not changed appreciably. (Siphon out the buffer from the cathodic (upper) tank & store it separately for reuse either in the cathodic or anodic chamber. Siphon out the buffer from the anodic (lower) tank & store it separately for reuse **only** in the anodic chamber.
3. Remove the binder clips.
4. Lift out the gel-sandwich & insert a gel sandwich opener (do not use a metal spatula as it will damage the plates) between the plates & prise them apart. Remove top plate & spacers from the sides.
5. Mark the orientation of the gel & carefully peel off the gel from the plate (pour water/buffer along the edge to ease the process).
6. Wash the apparatus immediately, with liquid detergent, & rinse with water. Dry & store in the boxes provided.

VIII Staining and de-staining the gel with CBB R-250:

1. Transfer the gel in staining tray containing staining solution.
2. Stain it for 60-90 min in CBB R-250 or in TechnoSource quick stain for 10-15 min for quick results
Note: Quick stain doesn't require destaining

IX De-staining the gel

1. Wash the gel (30 min/wash) in destaining solution till the background is clear.
2. Store the gel in 7% acetic acid