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**INSTRUCTION MANUAL
OF
VERTICAL ELECTROPHORESIS APPARATUS
(DUAL SIDED)**

MINIKIN

UNIDIRECTIONAL WITH INSITU CASTING

CAT. No. TVMNR-US1012 (OLD CAT. No. 01-00000)

MINIKIN

UNIDIRECTIONAL WITH CASTING STAND

CAT. No. TVMNR-UC1012 (OLD CAT. No. 01-00001)

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Dear Customer,

Congratulations on procuring the Minikin.

The Minikin is a versatile, robust and double-sided, compact vertical electrophoresis apparatus designed and manufactured in India.

Some points about the apparatus to keep in mind

- The apparatus is indigenously manufactured from high quality parts and is specifically designed to suit Indian laboratory conditions.*
- You can run either one, two, three or four gels simultaneously by using a single or dual gel sandwich on one or two sides.*
- The instrument has a very small footprint and hence can be run in a cold cabin.*
- Migration of sample in the wrong direction is prevented in the apparatus with unidirectional migration features.*
- Breakage resistant Teflon combs, spacers and fibre-glass notched plate help bring down the cost of spares and accessories.*
- There is no electrolytic corrosion of male connectors as the connectors are gold plated and the base is resin filled*
- Sharper bands achieved due to the thin glass plates.*

This vertical apparatus will give you many years of trouble free use. In any event that there is a problem our long warranty of 3 years covers all manufacturing defects.

We sincerely hope you that you gain as much satisfaction in using this apparatus as we achieved in designing and manufacturing it.

Regards,

The Production Team of TechResource

Precautions:

1. Use with care. Wash and rinse with soft water, air dry at room temperature & store covered.
2. Remove the CEU from the tank, wash and dry separately after every use.
3. The apparatus should **not be**:
 - a. Subjected to impact
 - b. Exposed to temperatures outside the range of 0 - 40°C
 - c. Handled with abrasive or sharp objects
 - d. Exposed to solvents/ acids which attack plastics
4. To avoid electrolytic corrosion, always dry the connecting pins before attaching the electrical cords
5. Please read the manual carefully before using the instrument.

Pre-installation check

- A. Check for any physical damage that may have occurred in transit
- B. Check if all the components are provided
- C. Functionality test
 1. Slide the CEU into the tank
 2. Add electrophoresis buffer till both electrodes are covered
 3. Using the connecting cords, connect the apparatus to a power supply.
 4. Set the power supply to 100V CV,
 5. The formation of bubbles at the electrodes indicates that the apparatus is functional

Components of the apparatus:

Sr. No.	Component	Cat. No.	Cat. No.
		01-00000	01-00001
1.	One tank	Yes	Yes
2.	One cathodic electrode unit (CEU)	Yes	Yes
3.	Two gaskets for CEU	Yes	Yes
4.	One Vented Lid	Yes	Yes
5.	One set of connecting cords	Yes	Yes
6.	One set of clamps (4 nos.)	Yes	Yes
7.	Four screws for clamps	Yes	Yes
8.	Four slotted spacers	Yes	No
9.	Four spacers (one end rounded)	No	Yes
10.	Two combs	Yes	Yes
11.	One set of glass plates [all ends square] (2 nos. notched & 2 nos . rectangular)	No	No
12.	One set of glass plate [with bottom ends rounded] (2 nos. notched & 2 nos . rectangular)	Yes	Yes
13.	One dual gel casting stand with for variable gel thickness and number, for agarose free sealing, fits 1-4 gels	No	Yes
14.	Two C shaped rubber gaskets each for single gel of 1.5 mm	No	Yes
15.	One spacer alignment tool	No	Yes
16.	One agarose dispenser	Yes	No
17.	One gel sandwich opener	Yes	Yes
18.	One clamp opener	Yes	Yes

Additional handy components that could be ordered:

1. A glass plate stand
2. Notched fibreglass plate (breakage resistant)
3. Different sizes of C shaped casting gaskets
4. Buffer savers
5. Penta-legged levelling table

Other items needed to perform a slab gel electrophoretic experiment:

- a. *To clean plates:* A liquid detergent, tap water and a squirt bottle containing distilled water.
- b. *To assemble the set-up:* Tissue and blotting paper, 10 mL pipette, agar (2%) in water.
- c. *To prepare the mixture for polyacrylamide gels:* Acrylamide stock soln., separating gel buffer stock soln., stacking gel buffer stock soln., TEMED, 10% ammonium persulphate/riboflavin, (ionic detergent / chaotropic agents), distilled water, 5 mL pipettes, 200 μ L and 20 μ L pipetting dispensers and their corresponding tips, levelling table, stoppered side-arm flask and vacuum pump.
- d. *To mark the various positions:* A glass-marking pen.
- e. *To prepare samples:* Polypropylene tubes, tracking dye (bromophenol blue) in appropriate buffer, boiling water-bath, tube rack (immersible in boiling water) and high-speed centrifuge fitted with appropriate rotor.
- f. *To load the samples:* Micropipette.
- g. *To separate the sample components:* A power supply minimum voltage – 200 V, minimum current – 200 mA.
- h. *To prepare the mixture for agarose/composite gels:* Agarose (preferably low gelling temperature), separating gel buffer stock soln., stacking gel buffer stock soln., GDW, stoppered flask, 250 mL beakers, 5 mL pipettes, thermometer (0 - 110°C), constant temperature regulated water-bath, hot plate or Bunsen burner.

ASSEMBLING THE APPARATUS—A SUMMARY

Cat. No. 02-00142 / 02-00143	Cat. No. 02-00144 / 02-00145
1. Slide the CEU into the tank, fasten in place by tightening the screw on the back side. 2. On the CEU, arrange the sandwich. The sandwich consists of the notched plate, spacers, and rectangular plate. 3. Insert the clamps along the edges of the plate and turn the screws clockwise to clamp the sandwich to the CEU (do not over-tighten). 4. Seal the bottom of the sandwich with 3–5 mL of molten agar/agarose. 5. Seal the sides by introducing hot agar/agarose into the slots of the spacers. 6. Pour the acrylamide mixtures in between the plates of the sandwich and insert the appropriate comb. 7. Once the gel has polymerized, remove the comb, fill the tanks with buffers, load the samples in the wells and apply electrovoltaic potential across the electrodes. 8. After the separation is done, stop the electrophoresis, disassemble the apparatus and treat the gels appropriately for staining / blotting / autoradiography.	1. Slide the CEU into the tank, fasten in place by tightening the screw on the back side. 2. Arrange the sandwich and place in the gasket provided. The sandwich consists of the notched plate with two bottom ends rounded, two spacers with one end rounded, and rectangular plate with two bottom ends rounded. Align the rounded ends such that they over lay each other. 3. Place the gasket containing the sandwich into the casting stand and clamp it. (The notched plate should be facing out.) 4. Pour the acrylamide mixtures in between the plates of the sandwich and insert the appropriate comb. 5. Once the gel has polymerized, disassemble the casting stand and remove the sandwich from the gasket. 6. Place the sandwich on the CEU with the notched plate facing in. 7. Insert the clamps along the edges of the plate and turn the screws clockwise to clamp the sandwich to the CEU (do not over-tighten). 8. Remove the comb, fill the tanks with buffers, load the samples in the wells and apply electrovoltaic potential across the electrodes. 9. After the separation is done, stop the electrophoresis, disassemble the apparatus and treat the gels appropriately for staining / blotting / autoradiography.

ASSEMBLING THE MINIKIN—A SUMMARY

- 1) Slide the CEU into the tank.
- 2) On the CEU, arrange the sandwich which consists of the notched plate, spacers, and rectangular plate on it.
- 3) Insert the clamps along the edges of the plate and turn the screws clockwise to clamp the sandwich to the CEU (do not over-tighten).
- 4) Seal the bottom of the sandwich with 4–6 mL of molten agar/agarose.
- 5) Seal the sides by introducing hot agar/agarose into the slots of the spacers.
- 6) Pour the acrylamide mixtures in between the plates of the sandwich and insert the appropriate comb.
- 7) Remove the comb, load the samples in the wells, fill the tanks with buffers and apply electrovoltaic potential across the electrodes.
- 8) After the separation is done, stop the electrophoresis, disassemble the apparatus and treat the gels appropriately for staining / blotting / autoradiography.

DETAILED PROCEDURE FOR ASSEMBLING THE MINIKIN.

I Cleaning the Apparatus:

- 1) Rinse all the items with tap (soft) water and shake off the excess water.
- 2) Wash the glass (and fibre-glass) plates thoroughly with detergent powder. (A shaving brush with flattened bristles is a very useful aid in cleaning.) Check if the water drains off evenly from the entire surface; else, clean those hydrophobic portions again. Finally, rinse the plates with distilled water and place them in the glass plate stand. (Note: It is necessary to clean the plates thoroughly to ensure that the gel adheres properly to the plates).

II Assembling the sandwich:

- 1) Slide the CEU along the guides on the inner sides of the tank. Ensure that the gasket is in the U-shaped groove and that the four feet rest on the tank.
- 2) Tilt the tank backwards by placing a buffer saver under the front edge so as to enable the plates to rest comfortably against CEU. (If the surface on which the unit is placed is too smooth, it may be necessary to place some blotting paper under the tank and the buffer saver.)
- 3) Place the notched plate against the gasket (notched side up; matt surface of fibre-glass plate facing outward).
- 4) Align the spacers vertically along the edges of the notched plate (shoulder of spacer facing inwards) while ensuring that their entire bottom edges rest on the platform of the tank.
- 5) Place the rectangular glass plate against the spacers. Align the entire sandwich with the CEU.
- 6) Fit the screws on the embossed side of the clamp strip. (Note: The threading is **left handed**.)
- 7) Position two clamps vertically along the edges of the sandwich (embossment of clamp on the top and facing outwards). With the thumb and forefinger of one hand (thumb up), gently turn the screw clockwise. To prevent the clamp-strip from moving, press it against the CEU with the thumb of the other hand, and at the same time push it downwards with the forefinger. Turn each screw alternately, until the clamps are just prevented from being lifted vertically up. (At this point, the pressure required to further turn the screw increases markedly.) Remove the buffer saver from under the tank. To secure the sandwich firmly, turn the screws by another quarter to half a turn. Check if the entire apparatus can be lifted off the table by the clamps. (Note: Do **NOT** use any gadget, such as the clamp-opener, to tighten the clamps, since excessive pressure on the notched plate will shorten its life. The threading of the screws can also give way.)
- 8) At this point check and ensure that: the shoulders of the spacers face inwards; that the spacers are vertically placed along the edges of the sandwich; that the entire bottom edge of the spacers rests on the platform of the tank; that the buffer savers can be placed in the tank; that the clamps rest on the platform of the tank; and that

the comb can be easily introduced into the sandwich so as to rest on the shoulders of the spacers. Loosen the clamps and make adjustments if necessary.

9) Assemble the second sandwich in a similar manner. (Note: If only one gel has to be cast, clamp the acrylic dam accessory on the other side of the CEU.)

10) With the aid of a glass-marking pen and a measuring ruler, mark the level to which the separating gel mixture has to be poured.

III Sealing the sandwich:

1) Place the apparatus on a flat and levelled surface. Melt the agar/agarose. Freshly prepared agar/agarose should be heated till the solution is clear.

2) Check if the agar/agarose sets firmly. To do so, transfer a small amount (0.1–0.2 mL) of the melted agarose onto a glazed tile / glass slide or table top. Allow to cool for 5 min. With the forefinger check that the consistency of the agar/agarose is firm. Do not use if it is runny or is of a mushy consistency. Prepare fresh sealant from a different batch/source.

3) To seal the bottom of the sandwich, transfer (with a pipette or the agarose dispenser) 4–10 mL very hot molten agar/agarose along the base of the sandwich, from the bottom of one clamp to the bottom of the other. Using the agarose dispenser, inject about 0.5 mL of very hot molten agar/agarose into the slot of each spacer. Some of the molten agar/agarose will drain out. Allow the agar/agarose to cool for 10–15 min. The agar/agarose fills the gap between the plates and solidifies. [Note: For a routine 10 %T acrylamide gel, about 4 mL of 1.2% agarose sealant solution is sufficient to support the weight of the liquid column at 25°C. When solutions with higher densities are used for polymerization, increase the agarose concentration and the amount of sealant appropriately. When the ambient temperature is higher than 30°C, it is necessary to pour (approximately 50 mL) chilled water in the lower tank after the agarose has set. This step ensures that the agarose remains firm during the polymerization of the acrylamide.]

4) It is essential that the agar/agarose solution be hot when the sides of the sandwich are being topped with agar/agarose. Re-heat the solution to about 70°C. Draw the hot solution into the dispenser (about 0.8 mL for 1.5 mm thick spacers). Position the end of the needle into the slot of the spacer with its tip aiming inwards and downwards. Inject the molten material rapidly, such that the stream of agar/agarose reaches the bottom and fills up the slot completely. If the solution is too hot, it could melt the bottom plug and cause a leak from the slot. Re-inject some more agar/agarose, after whatever material remaining at the bottom of the slot solidifies and cools. Sometimes bubbles are trapped in the slot; however, the sealing will still be effective.

IV Casting polyacrylamide slab gels:

1) Ensure that the apparatus is placed on a flat and levelled surface. For this purpose, a levelling table can be used (available as an accessory), or fill water in the lower tank, and adjust the unit until the water surface is parallel to the levelling line drawn on the transparent face of the lower tank.

2) Mix water (resistivity > 10 megohm), stock acrylamide and buffer solutions in a side-arm flask. Place the de-gassing stopper (available as an accessory) over the mouth of the flask and connect the side-arm to a vacuum pump. De-gas the mixture for 2–3 min. Add the catalyst (and a detergent, if desired) and mix by swirling. Add the initiator, mix, and transfer the mixture to the sandwich. Fill up to the mark on the glass plate (or fill to the top and introduce the comb if the stacking gel is not used). Keep the remaining mixture aside.

The user can select spacers made of either Polymethyl methacrylate (PMMA), Polytetrafluoroethylene (PTFE) or polycarbonate (PC). Spacers made of PMMA have to be handled carefully. However, it is one of the few plastics to which polyacrylamide adheres. PTFE and PC spacers, on the other hand, are breakage resistant but, as is the case with most plastics, polyacrylamide does not adhere to it. Hence, there is a channel of unpolymerized solution between the gel and the PTFE or PC spacer. The width of this channel is directly proportional to the oxygen content of the polymerization mixture (Cold solutions and deionized water are **richer** in dissolved oxygen than are warm solutions and fresh distilled water). Often the unpolymerized solution drains out when the separating gel is being washed. When the stacking gel mixture is poured into the sandwich, it drains down through this channel and can lead to poor well formation. A symptom of this malady is the drooping down of the dye-front along the edges of the gel. Hence, it is **essential** to de-gas the polymerization mixtures whenever PTFE or PC spacers are used.

- 3) Carefully overlay the acrylamide mixture with 0.5–1 mL of distilled water. (Note: If water-saturated butan-2-ol is used as an overlay for low %T gels, use **only** analytical or spectroscopic grade butanol. Butanol of lower purity contains contaminants which corrode the spacers.)
- 4) After 30 minutes, check if the mixture remaining in the side-arm flask has polymerized. If so, check if the contents of the sandwich have polymerized by tilting the apparatus sideways. After the mixture in between the plates has polymerized, invert the apparatus over a sink to drain out the overlay solution and the unpolymerised mixture.
- 5) Wash the top of the polyacrylamide gel with distilled water.
- 6) Repeat step 2 of section IV to polymerize the stacking gel. However, before adding the initiator, stand the MINIKIN on its side to drain out the water that has gathered on the surface of separating gel. Invert the MINIKIN and with an absorbent paper, blot the last drop which trickles down the edge of the spacer.
- 7) Add initiator to the stacking gel mixture, mix thoroughly, and immediately transfer the contents on the top of the separating gel. Introduce the comb and allow the gel to set. Avoid trapping bubbles under the teeth of the comb.
- 8) Mark dots (with a water-proof marking pen) on the glass plate, at the centre and the bottom of the wells to indicate their positions.
- 9) Check if the stacking gel remaining behind in the flask has polymerized.
- 10) To remove the comb, pour GDW over it (or fill the cathodic chamber with GDW), and while doing so pull it out slowly. Ensure that water fills the void in the wells.
- 11) Wash the wells with GDW. If the stacking gel is fragile use a syringe to wash the wells, else, add GDW to the wells with a pipette and then flick the water out by jerking the apparatus over a sink. Then, fill the wells with 1x stacking gel buffer.

V Casting agarose or polyacrylamide-agarose composite slab gels:

Gels containing agarose are fragile and adhere poorly to the plates. They also have a high coefficient of thermal expansion. When agarose-containing gels are held between two plates, they are not free to expand or shrink with changes in temperature, and being fragile will crack. Consequently, to prevent shrinkage after gelling, the grade of agarose used should be that which sets closest to ambient (low melting agarose is ideal). The electrophoresis should also be carried out at ambient temperatures. The agarose should be cooled to within 5–8°C of its setting temperature in the flask and then poured into a pre-warmed sandwich to minimize problems associated with shrinkage. In the case of composite gels, the initiator and catalyst should be added just before pouring the gel into the cassette, otherwise, at the high temperatures used to melt the agarose, the acrylamide will polymerize rapidly. For best results with composite gels, the agarose should gel first, and then the acrylamide should polymerize within its pores.

- 1) Ensure that the apparatus is placed on a flat and levelled surface away from any draught of air (such as that from an air-conditioner).
- 2) Heat water in a temperature controlled water-bath to $T^{\circ}\text{C}$. (T is the temperature that is 8°C above the gelling temperature of the agarose used.)
- 3a) To cast agarose gels, mix the required quantity of agarose, separating gel buffer stock solution, detergent (if desired), and GDW in a glass-stoppered conical flask.
- 3b) To cast polyacrylamide-agarose composite gels, mix the required quantity of agarose, separating gel buffer stock solution, acrylamide-crosslinker stock solution, detergent (if desired), and GDW in a glass-stoppered conical flask. (Catalyst and initiator are added later.)
- 4) Prepare the separating gel by heating the stoppered conical flask in a boiling water-bath till the solution turns clear, mix and continue heating it for another 5 min.
- 5) Cool the buffered separating gel solution to $T^{\circ}\text{C}$ by placing it in the temperature controlled water-bath. To prevent the flask from toppling, place it in a perforated plastic can (available as an accessory).
- 6) Heat distilled water (about 1 L) to 60–70°C. Adjust its temperature to $T^{\circ}\text{C}$ with cold distilled water. Switch off any air circulators in the vicinity of the apparatus and pour the $T^{\circ}\text{C}$ distilled water into the upper and lower tanks.
- 7a) To cast agarose gels, warm a 10 mL pipette by drawing hot water (60–70°C) into it. Draw about 15 mL of molten agarose separating gel solution (at $T^{\circ}\text{C}$) into the pipette, allow any bubbles to rise to the surface and then

transfer the molten material to the sandwich. (To prevent the agarose from cooling below $T^{\circ}\text{C}$, the stoppered flask should be immersed in a beaker containing warm water while it is being transported from the water-bath to the apparatus.) Fill the sandwich upto the mark on the glass plates.

7b) To cast polyacrylamide-agarose composite gels, add appropriate amounts of the catalyst and initiator to the warm, molten composite gel solution, mix thoroughly. Warm a 10 mL pipette as described in section V step 7a, and draw about 15 mL of molten composite separating gel solution (at $T^{\circ}\text{C}$) into the pipette, and then transfer the molten material to the sandwich. Follow precautionary steps as outlined in section V, step 7a.

8a) In the case of agarose gels, remove any air bubbles on the surface with the help of the agarose dispenser and a long hypodermic needle.

8b) In the case of composite gels, carefully overlay the mixture with 0.5–1 mL of warm ($T^{\circ}\text{C}$) distilled water.

9) From the tanks, siphon out the warm distilled water with the aid of a clean drainage tube. Do not drop any water into the sandwich.

10) Allow the gel to set firmly. Depending on the type of agarose used, the setting time will vary from 30 to 90 min.

11) Re-heat about 500 mL of the siphoned distilled water to 60–70°C.

12) Mix the required quantity of agarose, stacking gel buffer stock solution, detergent (if desired) and GDW in a glass-stoppered conical flask.

13) Prepare the stacking gel by heating the stoppered conical flask in a boiling water-bath till the solution turns clear, mix and continue heating it for another 10 min. A few drops of bromophenol blue dye may be added to the molten agarose such that it is light blue. (The colour will aid comb removal) Then cool the buffered agarose to $T^{\circ}\text{C}$ by placing it in the temperature controlled water-bath.

14) Adjust the temperature of the hot distilled water to $T^{\circ}\text{C}$ as done earlier.

15) Pour the warm ($T^{\circ}\text{C}$) distilled water into the upper tank.

16) Warm the tapered teeth agarose-comb by immersing it in water of $T^{\circ}\text{C}$.

17) As mentioned in step V-7, use a pre-warmed 5 mL pipette to transfer the molten stacking agarose to the sandwich so as to fill it up.

18) Introduce the pre-warmed agarose-comb into the sandwich.

19) Carefully siphon out the warm water from the upper tank and allow the gel to set.

20) Mark dots (with a water-proof marking pen) on the glass plate, at the centre and bottom of the wells to indicate their positions.

21) Comb removal is the most delicate operation. The agarose between the teeth and the plate hinders comb removal and leads to well- separator breakage. To free the teeth from the agarose, insert a mylar strip between the comb and the rectangular glass plate. The strip should not dip below the level of the wells. Pull the strip along the entire length of the comb. Repeat the process on the notched plate side. Squirt water/buffer over the comb while simultaneously pulling it upwards **very slowly**. Ensure that the comb does not move sideways, else the well-separators will break.

VI Loading and separating the samples:

1) Fill the cathodic chamber with appropriate tank buffer till just below the brim.

2) Fill the anodic tank with buffer upto the levelling line. Check if the unit is still levelled. If necessary, place the buffer savers in the lower tank; else, fill the anodic tank with buffer upto the top of the 'MINIKIN' embossment. The ammonium persulphate used as the initiator of acrylamide 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 and damage (oxidatively) the proteins. Some of the ways by which this damage can be minimized are: 1) Use riboflavin as the initiator in the stacking gel. Load the samples and perform the electro-phoresis in safe/subdued light; ii) In the sample buffer, use reducing agents such as 2-mercaptoethanol or dithiotreitol at high concentrations; iii) Electrophoretically remove the persulphate (pre-run) before the sample is loaded. For this, a small amount of 1x loading buffer is added to one of the wells, and the dye is forced to migrate under the influence of a voltage gradient until it reaches exactly midway into the stacking gel. The electrophoresis is stopped and the samples are loaded immediately as described below.

3) Carefully introduce the (particulate free) samples into the wells. For best results, ensure that all the wells are filled to the same level, and that the ionic strength (salt concentration) of all the samples is the same. To the unused wells, add 1x loading buffer whose ionic composition is made the same as that of the samples.

When a continuous buffer system is employed, the resolution is critically dependant on the manner in which the samples are loaded. Lower the tip of the sample applicator such that it is 1–2 mm above the bottom of the well. Carefully load (about 10–15 $\mu\text{L}/7.5 \text{ mm}^2$ of well bottom surface) a dense sample, so as to avoid mixing. For continuous buffer systems, do **NOT** introduce sample at the top of the well and allow it to sink to the bottom.

- 4) Blot out any liquid that may be around the male connectors on the unit (CEU) to prevent their electrolytic corrosion.
- 5) Cover the apparatus with its lid.
- 6) Connect the electrical cords according to the colour codes (red to orange and black to black) and operate under conditions of constant current (In case a current does not flow through the apparatus, check the connections; for, the MINIKIN has been fitted with polarity-sensing circuit which prevents the passage of current in the reverse direction).
- 7) When running dual 10 %T polyacrylamide gels upto 1.5 mm thick in a refrigerator, use a maximum of 20 mA/cm² of gel surface area (thickness x width). When single gels are being run, 26 mA/cm² of gel surface can be used. (For a 1.5 mm thick, 10 cm wide gel, use 60 mA for dual gels and 40 mA in case of single gels).
- 8) As agarose gels shrink or swell (and consequently crack) with changes in temperature, do not cool agarose gels or polyacrylamide-agarose composite gels below room temperature. Also, do not allow the temperature to rise during electrophoresis. Hence, when operating under conditions of constant current, do not pass more than 16 mA/cm² of gel surface area in the case of dual gels and 20 mA/cm² of gel surface area in the case of single gels.

VII Dismantling the sandwich and removal of the polyacrylamide gel:

- 1) When the dye-front is about 5 mm from the bottom, the electrophoresis is complete. If there are differences in the length/composition of the gels on the two sides of the CEU, then there will be a difference in the rate of migration of the dye-front. To stop further migration of the dye-front that has already entered the agar/agarose, slip a flat object under that side (the side where the dye has entered the agar) so as to tilt the tank backwards. Ensure that the buffer in the upper tank makes contact only with the gel whose dye-front has lagged behind. Continue electrophoresis till its dye-front enters the agar.
- 2) At the end of the run, switch off the power supply, disconnect the electrical cords and remove the lid.
- 3) Siphon out the buffer from the cathodic (upper) tank and store it separately for reuse (either in the cathodic or anodic chamber), if its pH has not changed appreciably.
- 4) Siphon out the buffer from the anodic (lower) tank and store it separately for reuse (**only** in the anodic chamber) if its pH has not changed appreciably.
- 5) Remove the buffer savers and rinse them with water.
- 6) Tilt the tank backwards by placing a buffer saver under one side. Loosen the clamps by turning the screws anti-clockwise (use the clamp-opener if necessary). Remove the clamps and rinse them with water.
- 7) Lift out the gel-sandwich and place it horizontally on a sheet of paper. Pull out the spacers from the sides and discard the agarose in the dustbin.
- 8) Insert a spatula between the plates (near, but not at, the lower corner) and prise them apart.
- 9) Mark the orientation of the gel and carefully peel off the gel from the plate. The polyacrylamide gel attaches itself firmly to the fibre-glass plate. To peel gel from this plate, insert the gel-detacher under the gel, and while doing so pour water/buffer along its edge. Slide the gel-detacher towards the opposite edge, and pour water/buffer behind the gel-detacher to ensure that the gel does not re-adhere to the plate.
- 10) Wash the plates immediately, before any adhering pieces of gel dry up on it. Dry the plates and store them in the storage box provided.
- 11) Wash the CEU and flick off the excess water.
- 12) Scoop out the agar from the tank and discard it into a dustbin. Clean the tank in the sink. Ensure that the small pieces of agar do not get washed down the drain. Flick off the excess water.
- 13) Wash the clamp-strips, screws, spacers, etc., thoroughly with water. Dry and store them in the boxes provided.
- 15) Rinse the lid and dry it. Slide the dry CEU into the dry tank, place the lid over it and store it along with the connecting power cord in the container provided.

VIII Dismantling the sandwich and removal of the agarose or composite gels:

- 1) Follow protocol as described in section VII, steps 1–6.
- 2) Place the tank with its (clear) side on the table such that the sandwich to be removed is in a horizontal position and on the top. To separate the sandwich from the sealing agarose at the bottom of the tank, grip the plates and pull it upwards, till the glass plate touches the C-shaped gel-strip on the base of the tank. If the plates are removed vertically as is the case with polyacrylamide gels, there is a likelihood of only an empty sandwich being drawn up. The agarose/composite gel, which binds poorly to the plates, can slip out of the sandwich and remain behind in a collapsed state in the tank.
- 3) Always maintain the sandwich in the horizontal position. Place the gel-sandwich horizontally on the table. Pull out the spacers from the sides.
- 4) Insert a spatula between the plates (near, but not at, the lower corner) and prise them apart. Remove the top plate.
- 5) Mark the orientation of the gel and carefully place the plate with the gel on it, in the staining tray. Add fixative/water to the tray and carefully slide the gel off the plate.
- 6) Do not attempt to pour out solutions from the tray, as the gel can slide out and attempts to restrain it may cause breakage of the gel. Use a pipette, or siphon out the solutions, instead.
- 7) To clean the apparatus, follow protocol as described in section VII, steps 10–15.

SOME NOTES FOR CONVENIENCE

1. Preparing acrylamide stock solution:- Acrylamide and bisacrylamide are neurotoxins whose effects are cumulative. Exercise due caution while preparing and handling the solutions containing these substances. Assume 1 g of acrylamide occupies 1 mL. Warm the required amount of water in a beaker, introduce a PTFE-coated spin-bar and place on a magnetic stirrer. Weigh out bisacrylamide and add it to the stirring water. (Avoid drafts of air while weighing and transferring bis-acrylamide, since it is light and thus can be wafted off the paper on which it is weighed). Adjust the speed of the stirrer to ensure the floating powder is drawn under the water. After the bis-acrylamide has dissolved (the solution should be clear; else, use a new lot), add the acrylamide and continue stirring. The dissolution of acrylamide is endothermic in nature and it may be helpful to heat the solution. Filter and store at 4°C. The conductivity of a 10% solution should be < 5 $\mu\text{S}/\text{cm}$ and $A_{420} < 0.01$. If solution has been stored for a long time, it may be necessary to add 1 g Amberlite MB-1 monobed resin per 100 mL acrylamide stock solution to remove the acrylic acid formed.

2. Preparing sodium lauryl sulphate (SDS) stock solution:- Dissolve at 42°C, avoid foaming. **Do NOT** autoclave SDS. Normally stored at room temperature. In case the solution is stored at 4°C, warm it to dissolve the crystallized SDS and then use it.

3. Store ammonium persulphate (APS) in a desiccator. Powder should be free flowing and it should show strong effervescence while getting dissolved. Best used fresh. However, solutions can be stored in air-tight vials for upto 1 week at 4°C.

4. Use gloves while handling TEMED. Because of its obnoxious smell, open the bottle in a fumehood. It is convenient to prepare a 10% solution in GDW in the fumehood and use it.

5. Keep the gelled agarose in 15 mL aliquots. If the agarose is melted time and again, the concentration increases and it tends to clog the slots of the spacers while sealing.

Caution: When the ambient temperature is below 25°C, the molten agarose in the flask will get cold during the time it takes for the agarose at the bottom of the sandwich to solidify. Such cool molten agarose tends to solidify before it reaches the bottom of the slots in the spacers. To keep the agarose hot, keep the flask/vial in a beaker of hot water. Also, do not keep the hot molten agarose on (black) stone-topped tables. The stone conducts away the heat rapidly and cools the agarose.

6. DO NOT polymerize both the separating and the stacking gels for storage overnight, since the pH gradient between them would get lost by diffusion in the storage period. If you cast the separating gel overnight, overlay with 1 x separating gel buffer.

Note: This practice may cause the samples in the border lanes to migrate slower than those in the other lanes.

7a. Good gels are formed when the polymerization occurs in 30–45 min. The polymerization of acrylamide is an exothermic process, and at high %T, the heat produced raises the temperature, and consequently, speeds up the process. When these hot gels cool, they shrink and get distorted. Hence, if the acrylamide polymerizes in less than 30 min, either maintain the temperature of polymerization at 25°C by filling the upper and lower tanks of the MINIKIN with water, or lower the concentrations of the catalyst and/or initiator so as to complete the cross-linking in the above-mentioned time band.

7b. In the various protocols listed in this manual, the amounts of APS (initiator) and TEMED (catalyst) are appropriate for polymerization at 25°C. In case the room temperature is higher, decrease the quantity of these compounds, or fill water at 25°C in the tanks, so as to ensure that the gel polymerizes in 30–45 min.

7c. As a corollary to the points mentioned in 7a and 7b, the concentration of the catalyst and/or initiator would have to be increased when the room temperature or %T is low.

8. When urea is used as a denaturant, do not store urea-containing solutions since cyanate ions are formed. Preferably use primary amines (such as Tris) for buffers, as they combine with cyanate and consequently prevent it from reacting with proteins.

9. Glycerol is often added to the stacking gel at a final concentration of 5%. This practice results in sharper bands, especially if glycoproteins are being separated.

10. When CTAB is used as detergent for separation of proteins in a cationic state, use riboflavin instead of persulphate to polymerize the gel as the latter precipitates the detergent.

11. For best resolution, run SDS gels at 10°C. This can be achieved by placing the apparatus on a levelled surface in a refrigerator.

12. *Preparing Coomassie blue staining solution*:- Dissolve *Coomassie blue* in methanol. Then add water and acetic acid. Ensure that the acetic acid is not contaminated with metal ions (*e.g.*, Al^{3+} from the cap of the acetic acid bottle), the presence of which can cause the SDS to precipitate in the gel turning it white.

13. *Protein estimation by staining*:- Coomassie blue R poorly stains fibrous proteins and glycoproteins. Hence, such proteins may be underestimated by this staining procedure.

14. *Reuse of staining solution*:- a) It is good practice to immerse SDS-containing gels in 10% acetic acid or 12.5% TCA prior to immersing in staining solution. This step both fixes the protein and causes the SDS to leach out. Accumulation of SDS in the staining solution can lead to loss of sensitivity. b) With repeated use of staining solution, its acetic acid and methanol concentrations decrease. Consequently, proteins (even those previously fixed) could diffuse out. Hence, add fresh acetic acid and methanol to the staining solution periodically.

15. *Reuse of de-staining solution*:- Destaining solution is expensive. It can be reused by decolorizing it with activated charcoal.

16. *Pre-stained protein markers*:- Never use pre-stained markers to determine the molecular-weight of proteins, as the dye molecules change their M.W. significantly and unpredictably. Use them only to follow the course of electrophoresis and protein transfers. Stock solutions of pre-stained proteins should be stored frozen. They should be used immediately after they are diluted in loading buffer, as the dye tends to dissociate from them upon prolonged incubation.

17. Polyacrylamide gels are defined in terms of %T and %C. Where %T is the percentage of total acrylamide concentration (acrylamide and bis-acrylamide) and %C is the percentage of cross-linker (bis-acrylamide) in the total weight of the acrylamide. The gel concentration recommended for separating proteins of different masses is as follows:

Gel conc. %T	$M_r \times 10^3$
3–5	>100
5–12	20–150
10–15	10–80
>15	<15

Chemicals required for SDS Electrophoresis and staining of the gel.

- 1) Tris base [Tris]
- 2) Glycine (AR)
- 3) HCl (GR) (store in a fume hood)
- 4) Acrylamide (Neurotoxin, Handle with care)
- 5) Bisacrylamide (Neurotoxin, Handle with care)
- 6) TEMED (store at 4°C)
- 7) Ammonium persulfate [APS] (store in a desiccator)
- 8) Glycerol (GR or Excelsar)
- 9) 2-mercaptoethanol [2-ME] (store at 4°C)
- 10) Sodium dodecyl sulfate [SDS]
- 11) Glass double distilled water [GDW]
- 12) Low EEO Agarose
- 13) Methanol (LR or SQ) (store in a fume hood)
- 14) G. Acetic acid (GR or Excelsar) (store in fume hood)
- 15) Coomassie blue R-250 (Sigma, Fluka, Merck, SRL)

The water (GDW) used to prepare solutions should have a resistivity of more than one megohm. All solutions should be filtered and stored.

Acrylamide stock solution (30 %T, 2.7 %C)

Warm (50°C) GDW	65	mL
Bisacrylamide	800	mg
Dissolve		
Acrylamide	29.2	g
Dissolve		

Add GDW to make the volume up to 100 mL.

When the acrylamide concentration of the gel needed to separate the bio-molecules is greater than 20 %T, then a stock solution with 40 %T, 2.7 %C would have to be prepared.

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

GDW	30	mL
Tris	9.08	g

Dissolve

3 N HCl to pH 8.8 (about 4 mL)

Add GDW to make the volume up to 50 mL.

When the acrylamide concentration of the gel needed to separate the bio-molecules is greater than 20 %T, then an 8× Separating gel Buffer would have to be prepared.

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

GDW	35	mL
Tris	3.0	g

Dissolve

3 N HCl to pH 6.8 (about 8 mL)

Add GDW to make the volume up to 50 mL.

3 N H₃PO₄ is sometimes used instead of 3 N HCl.

20% SDS solution

Warm (50°C) GDW	80	mL
SDS	20	g

Dissolve (avoid foaming)

Do not store in plastic (LDPE) containers.

10% Ammonium persulphate (prepare on the day of use or a day before)

APS	100	mg
GDW	1	mL

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

Note: Highly acid systems do not gel well with APS.

CTAB is precipitated by APS.

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

Tris	3.0	g
Glycine	14.4	g
GDW	970	mL

Dissolve

20% SDS soln. 5 mL

Add GDW to make the volume up to 1000 mL.

Tracking dye stock solution

Methanol	10	mL
Bromophenol blue	50	mg
Dissolve		
GDW	90	mL

Use 1 µL/50 µL of sample.

Methylene Green, Methylene Blue or Pyronine Y can also be used as tracking dyes.

2× Loading buffer

4× Stacking gel buffer	2.5	mL
Glycerol	4.0	mL
Tracking dye stock solution	0.5	mL
Mix		
20% SDS soln	2.0	mL
Mix (avoid foaming)		
2-ME	1.0	mL
Mix (avoid foaming)		

Coomassie blue R-250 staining solution

Methanol	500	mL
Coomassie blue R-250	1.0	g
Dissolve		
Glacial acetic acid	100	mL
GDW	400	mL

Filter through Whatman #1 and store in a glass-stoppered / plastic-capped bottle. Keep away from metals.

Initial destaining solution (40% methanol, 10% acetic acid)

Methanol	800	mL
GDW	1000	mL
Glacial acetic acid	200	mL

Wash the gel thrice (30 min/wash) in initial destaining solution and then destain in subsequent destaining solution.

Subsequent destaining solution (5% methanol, 7% acetic acid)

Methanol	125	mL
GDW	2200	mL
Glacial acetic acid	175	mL

Convenient staining solution

Coomassie blue R-250 staining solution	8	mL
Initial destaining solution	192	mL

When gels are incubated in this solution, the proteins take up the stain while the background (gel) is lightly stained. The background can be decolorized with the Subsequent destaining solution. In an hour, bands containing 2 µg protein are visible, and upon overnight incubation, bands containing as little as 200 ng of protein can be detected.

Stained gel storage solution (7% acetic acid)

GDW	93	mL
Glacial acetic acid	7	mL

Preparation of 30 mL (denaturing) separating gel at 25°C.

Add the following solutions to a 250-mL thick-walled side-arm flask.

%T Acrylamide stock soln α mL where α = required % of separating gel.

4× Separating gel buffer	7.5	mL
GDW	22 - α	mL

Mix, filter and de-gas.

20% SDS solution	150	µL
TEMED	15	µL

Mix. Ensure that an aliquot of GDW (for overlay), a 1 mL and a 10 mL pipette are at hand.

10% APS	150	µL
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Mix and transfer to sandwich with a 10 mL pipette (approx 14 mL for a 9 x 10 cm separating gel). Introduce the acrylamide mixture along the centre of the rectangular plate; not along the sides of the spacers.

Carefully overlay GDW on the acrylamide mixture using a 1 mL pipette. Introduce the water along the trail of the acrylamide with the pipette inclined at 10–15° from the horizontal. The gel should polymerize in 30–45 min. If the gel polymerizes in more or less time, adjust the TEMED and/or APS concentrations appropriately.

Preparation of 10 mL (denaturing) stacking gel.

Add the following solutions to a 50-mL flask.

Acrylamide stock soln	1.34	mL
4× Stacking gel buffer	2.5	mL
GDW	6.0	mL

Mix, filter and de-gas.

20% SDS solution	50	µL
TEMED	10	µL

Mix. Ensure that the combs and a 5 mL pipette are at hand.

10% APS	50	µL
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Mix and transfer to sandwich with a 5 mL pipette. Insert the comb. Avoid trapping bubbles under the teeth of the comb. In case a film of polyacrylamide forms between the teeth of the comb and the plates, check the quality and quantity of the TEMED and APS used.

BUFFERS FOR DISCONTINUOUS SYSTEMS.

The advantage of discontinuous buffer systems is that they help concentrate the samples loaded, by stacking the bio-molecules. In some instances, such a concentration of the sample may cause some of its constituents to precipitate in the well. If such is the case, vertical bands of protein (streaking) will be present in the lanes.

As the persulphate may react with the bio-molecules being separated, stacking gels often use riboflavin as the initiator. In the separating gel, the persulphate ion moves ahead of the dye-front.

Recipe I

(pH during stacking 7.0 and, during separation 8.0. Useful for separating, in an active state, those enzymes which are inactivated above pH 8.)

4× Separating gel buffer (283 mM Tris-Cl; pH 7.5)

GDW	35	mL
Tris	1.71	g

Dissolve

1 N HCl to pH 7.5 approx 12 mL

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 2.7 %C, TEMED at 20 µL/30 mL, 10% APS at 20 µL/30 mL.

4× Stacking gel buffer (0.205 M Tris-PO₄; pH 5.5)

GDW	35	mL
Tris	1.24	g

Dissolve

3 N H₃PO₄ to pH 5.5 approx 9.7 mL

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 2.7 %C, 1% riboflavin at 15 µL/30 mL.

Tank buffer (8.26 mM Tris, 30 mM diethylbarbiturate; pH 7.0)

Tris	1	g
Diethylbarbituric acid	5.52	g
GDW	980	mL

Dissolve

Add GDW to make the volume up to 1000 mL.

Recipe II

(pH during stacking 8.3, and during separation 9.5. Useful for separating neutral and acidic proteins which are not affected by basic pH.)

4× Separating gel buffer (1.51 M Tris-Cl; pH 8.9)

GDW	30	mL
Tris	9.15	g
Dissolve		
3 N HCl to pH 8.9	approx	4 mL

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 5 %C, TEMED at 10 µL/30 mL, 10% APS at 20 µL/30 mL.

4× Stacking gel buffer (236 mM Tris-PO₄; pH 6.9)

GDW	35	mL
Tris	1.43	g
Dissolve		
1 N H ₃ PO ₄ to pH 6.9	approx	6.4 mL

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 20 %C, 1% riboflavin at 15 µL/30 mL.

Cathodic tank buffer (4.95 mM Tris, 38.4 mM glycine; pH 8.3)

Tris	0.6	g
Glycine	2.88	g
GDW	990	mL
Dissolve		

Add GDW to make the volume up to 1000 mL.

Anodic tank buffer (63 mM Tris-Cl; pH 7.5)

GDW	940	mL
Tris	7.63	g
Dissolve		
1 N HCl to pH 7.5		

Add GDW to make the volume up to 1000 mL.

Recipe III

(pH during separation 9.5. Used in the separation of SDS-denatured membrane proteins.)

4× Separating gel buffer (1.7 M Tris-Cl; pH 9.18)

GDW	30	mL
Tris	10.29	g
Dissolve		
1 N HCl to pH 9.18	approx	6 mL

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 0.9 %C, TEMED at 45 µL/30 mL, 10% APS at 150 µL/30 mL.

4× Stacking gel buffer (216 mM Tris-SO₄; pH 6.1)

GDW	35	mL
Tris	1.31	g

Dissolve

1 M H₂SO₄ to pH 6.10 approx 5.3 mL

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 6.25 %C, TEMED at 45 µL/30 mL, 10% APS at 150 µL/30 mL.

Cathodic tank buffer (41 mM Tris, 40 mM borate; pH 8.64)

Tris	4.97	g
Boric acid	2.48	g
GDW	980	mL
Dissolve		
20% SDS	5	mL

Add GDW to make the volume up to 1000 mL.

Anodic tank buffer (424 mM Tris-Cl; pH 9.18)

GDW	900	mL
Tris	51.45	g
Dissolve		
3 N HCl to pH 9.18	approx	10 mL

Add GDW to make the volume up to 1000 mL.

Recipe IV

(Useful for separating low molecular mass polypeptides, i.e., those less than 20 kDa.)

4× Separating gel buffer (3 M Tris-Cl; pH 9.3)

GDW	25	mL
Tris	18.17	g
Dissolve		
4 N HCl to pH 9.3		

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 0.5 %C and 10% glycerol in gel.

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

GDW	35	mL
Tris	3.0	g
Dissolve		
3 N HCl to pH 6.8		

Add GDW to make the volume up to 50 mL.

During polymerization, use bis-acrylamide at 4.8 %C and 10% glycerol in gel.

Tank buffer (50 mM Tris-Cl, 380 mM glycine; pH 8.5)

Tris	6.05	g
Glycine	28.54	g
GDW	950	mL
Dissolve		
1 N HCl to pH 8.5		

Add GDW to make the volume up to 1000 mL.