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

MITOKIN

UNIDIRECTIONAL WITH INSITU CASTING

CAT. No. TVMTR-US1012 (OLD CAT. No. 06-00122)

MITOKIN

UNIDIRECTIONAL WITH CASTING STAND

CAT. No. TVMTR-UC1012 (OLD CAT. No. 06-00124)

MITOKIN -BD

BI-DIRECTIONAL WITH INSITU CASTING

CAT. No. TVMTR-BS1012 (OLD CAT. No. 06-00123)

MITOKIN -BD

BI-DIRECTIONAL WITH CASTING STAND

CAT. No. TVMTR-BC1012 (OLD CAT. No. 06-00125)

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Dear Customer,

Congratulations on procuring the Mitokin.

The Mitokin is a versatile, robust and single-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 gel or two gels simultaneously by using a dual gel sandwich.*
- 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 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, fasten in place by tightening the screw on the back side
 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

Essential components of the apparatus:

Sr. No.	Component	Cat. No.			
		06-00122	06-00123	06-00124	06-00125
1.	One tank with attached anodic electrode.	Yes	Yes	Yes	Yes
2.	One cathodic electrode unit (CEU)	Yes	Yes	Yes	Yes
3.	One gasket for CEU	Yes	Yes	Yes	Yes
4.	One Lid	Yes	Yes	Yes	Yes
5.	One set of connecting cords	Yes	Yes	Yes	Yes
6.	One set of clamps	Yes	Yes	Yes	Yes
7.	Two screws for clamps	Yes	Yes	Yes	Yes
8.	Two slotted spacers	Yes	Yes	No	No
9.	Two spacers (one end rounded)	No	No	Yes	Yes
10.	One comb	Yes	Yes	Yes	Yes
11.	One set of glass plates [all ends square] (Notched& Rectangular)	Yes	Yes	No	No
12.	One set of glass plate [with bottom ends rounded] (Notched& rectangular)	No	No	Yes	Yes
13.	One spacer alignment tool	No	No	Yes	Yes

Additional handy components provided:

1. One agarose dispenser (Only with Cat. No. 06-00122 & 06-00124).
2. A gel sandwich opener.
3. A glass plate stand.

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.
- 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. 06-00122 / 06-00123	Cat. No. 06-00124 / 06-00125
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.

DETAILED PROCEDURE FOR ASSEMBLING THE APPARATUS.

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 mild 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. Clean the gasket of the casting stand (If you are using it). 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 for Cat No. 06-00122 and 06-00123 (models without casting stand):

For assembling the sandwich for Cat. No. 06-00124 and 06-00125 go to IV below

- 1) Place the tank on a levelled surface with the anode pin (on red handle) facing oneself.
- 2) Loosen the screws on the clasping extension provided on the back-side of the CEU. Hold the CEU with the gasket (in U groove) facing oneself. Place the CEU in the tank such that the back wall of the tank is between the main part of the CEU and the extending clasp, provided for holding it in place. Tighten the clasping screws, so that the CEU is secured to the tank.
- 3) Tilt the tank backwards by placing a small object 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 object.)
- 4) Place the notched plate against the gasket (notched side up; matt surface of fibre-glass plate facing outward).
- 5) 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.
- 6) Place the rectangular glass plate against the spacers. Align the entire sandwich with the CEU.
When two gels have to be cast, place a notched glass plate instead of the rectangular plate; then position another set of spacers and align them as described in step II.5. Place the rectangular plate in position as described II.6.
- 7) Hold the clamp with the protruding end downwards and facing you, and the side with screws away from you. Hold the clamp at an angle with the top end tilting outwards. Insert the protruding end of the clamp into the tank, and gently slide the top part of the clamp into place such that the sides of the assembled sandwich and CEU wall are within its grasp. Repeat the process with the other clamp. Tighten the screws at the back of the clamp in an alternate manner, so that the sandwich is held firmly in place. **NOTE:** Do not tighten the screw on one clamp completely whilst the other one is completely loose, else, the other edge sandwich will fan out and when the other clamp is fitted and tightened the notched glass plate could break. Do not overtighten the screws.
- 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 clamps rest on the sides 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) 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 and also mark the rectangular plate to indicate the bottom and centre of each well. This last step is essential to aid loading of samples when a fibre-glass plate is used or when samples are simultaneously being separated on two gels.

III Sealing the sandwich:

- 1) Place the apparatus on a flat and levelled surface and tilt the tank backwards by placing a small object under the front edge. 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) 3–5 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 0.4 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 2 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 0.5 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 Assembling the sandwich for Cat No. 06-00124 and 06-00125:

For assembling the sandwich for Cat. No. 06-00122 and 06-00123 go to II above.

If the sandwich has already been assembled go to Section V below.

- 1) Place the tank on a levelled surface with the anode pin (on red handle) facing oneself.
- 2) Loosen the screws on the clasp extension provided on the back-side of the CEU. Hold the CEU with the gasket (in U groove) facing oneself. Place the CEU in the tank such that the back wall of the tank is between the main part of the CEU and the extending clasp, provided for holding it in place. Tighten the clasp screws, so that the CEU is secured to the tank.
- 3) Place the casting stand on a levelled surface and slide the clamps open, such that the front transparent plate is level with the table.
- 4) On a clean surface place the rectangular glass plate with the rounded ends towards you. On the edges of this plate place the spacers with the rounded bottom ends overlapping the rounded ends of the glass plate. Place the notched plate against the spacers, again making sure that the rounded ends overlap the rounded ends on the spacers.
- 5) Place the entire sandwich within the gasket. Make sure the sandwich is well placed in the gasket. Slide the spacer alignment tool into the sandwich and once the spacers are aligned remove the spacer alignment tool

When two gels have to be cast, place a notched glass plate instead of the rectangular plate; then position another set of spacers and rectangular plate as described in step IV.5.

- 6) Place the gasket along with the sandwich in the casting stand and shut the front plate and slide the clamps into their slots. Thus the front plate now presses the gasket and seals the sandwich from three sides.
- 7) Introduce the comb into the sandwich and 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 and also mark the plate to indicate the bottom and centre of each well. This last step is essential to aid loading of samples when a fibre-glass plate is used or when samples are simultaneously being separated on two gels.
- 8) Remove the comb and cast the polyacrylamide gel (section V).
- 9) Once the gel is cast disassemble the casting stand and remove the gasket with the sandwich. Carefully pry the gasket away from the sandwich and place the sandwich on the CEU with the notched side facing inwards.
- 10) Hold the clamp with the protruding end downwards and facing you, and the side with screws away from you. Hold the clamp at an angle with the top end tilting outwards. Insert the protruding end of the clamp into the tank, and gently slide the top part of the clamp into place such that the sides of the assembled sandwich and CEU wall are within its grasp. Repeat the process with the other clamp. Tighten the screws at the back of the clamp in an alternate manner, so that the sandwich is held firmly in place. **NOTE:** Do not tighten the screw on one clamp completely whilst the other one is completely loose, else, the other edge sandwich will fan out and when the other clamp is fitted and tightened the notched glass plate could break. Do not overtighten the screws.

V Casting polyacrylamide slab gels:

- 1) Ensure that the apparatus/ casting stand is placed on a flat and levelled surface. For this purpose, a levelling table can be used (available as an accessory).
- 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 PMMA or polycarbonate (PC). Spacers made of PMMA have to be handled carefully. However, it is one of the few plastics to which polyacrylamide adheres. Polycarbonate 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 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 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, invert the apparatus to drain out the water that has gathered on the surface of separating gel, and using 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) Check if dots (with a water-proof marking pen) are marked on the glass plate, at the centre and the bottom of the wells to indicate their positions. If not do so.
- 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.

VI Loading and separating the samples:

- 1) Fill the cathodic chamber with appropriate tank buffer till just below the brim.
- 2) Add buffer to the anodic tank such that it is above the level of the bottom of the cathodic tank. Check if the unit is still levelled.

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: i) Use riboflavin as the initiator in the stacking gel. Load the samples and perform the electrophoresis 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. [When samples are being separated on two gels, load the back gel first. Some users lift the comb of the front gel only half way up (and not fully out) when the wells in the back gel are being loaded so that they do not load the front wells accidentally.] 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 1× 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 5–10 $\mu\text{L}/7.5 \text{ mm}^2$ 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 apparatus has been fitted with polarity-sensing circuit which prevents the passage of current in the reverse direction).
- 7) When running a 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). (For a 1.5 mm thick, 8 cm wide gel, use a maximum of 24 mA).

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.
- 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) Tilt the tank backwards. Loosen the clamps by turning the screws anti-clockwise. Remove the clamps and rinse them with water.
- 6) Lift out the gel-sandwich and place it horizontally on a sheet of paper. Insert a gel sandwich opener/ spatula between the plates (near, but not at, the lower corner) and prise them apart. Remove top plate and then spacers from the sides and discard the agarose in the dustbin.
- 8) 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 a gel detacher under the gel, and while doing so pour water/buffer along its edge. Slide a gel detacher on the opposite edge, and pour water/buffer behind the spacer to ensure that the gel does not re-adhere to the plate.
- 9) Wash the plates immediately, before any adhering pieces of gel dry up on it. Dry the plates on glass plate stand and store them.
- 10) Wash the CEU and flick off the excess water.
- 11) 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.
- 12) Wash the clamps, screws, spacers, etc., thoroughly with water. Dry and store them in the boxes provided.
- 13) 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.

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

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

13. *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.

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

15. *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.

16. 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