ST. XAVIER'S COLLEGE, MAPUSA GOA

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DEPARTMENT OF MICROBIOLOGY

Standard Operating Procedures

DBT STAR COLLEGE SCHEME

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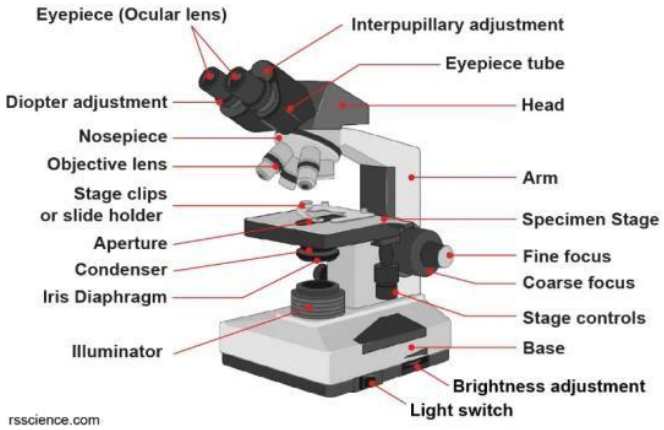
SOP 1: TRINOCULAR MICROSCOPE

Before using a microscope, be familiar with its structural and optical parts.



Microscopes are to be handled with utmost care

- Always carry a microscope with both hands.
- Remove the microscope from the cabinet, with one hand holding the arm of themicroscope and the other hand supporting the base.
- Place the microscope on the table with the arm of the microscope towards you andthe stage directed away from you.

STEPWISE GUIDE TO USE A MICROSCOPE

1) **Check the illumination.**

a) Rotate the nose piece and put the **low power objective** (10 X) in alignment (it willclick once in position).

b) Open the iris diaphragm fully.

c) Adjust the mirror to face the light to get maximum illumination. If the microscope has built-in light bulb, switch on the light.

2) **Mount the slide**

a) Using the coarse adjustment knob lower the stage to its lowest point before

placingthe slide on it.

b) Place the slide on the stage and fasten it with the stage clips.

c) Look at the objective lens and the stage from the side (look sideways not down themicroscope) and turn the coarse adjustment knob so the stage moves upwards. Move it up as far as it goes but without letting the objective touch the slide.

3) Focus the slide

For **Low power objective lens (10X)**

a) Look through the eyepiece and move the fine adjustment knob such that theobjective lens moves away from the slide, until the image comes into focus.

b) Adjust the condenser and light intensity for maximum amount of light.

c) Move the stage using stage control knob such that the sample is in the centre of the field of view.

d) When you see a clear image of the specimen record your observations OR changeto higher power lens.

For **High Power objective lens(40X)**.

a) Gently turn the revolving nosepiece to high power lens (40X), see that it clicks intoposition.

b) Look through the eyepiece and move the fine focus such that the objective lensslowly moves away from the slide, until the image comes into focus.

c) Readjust the condenser and light intensity if needed.

d) When you see a clear image of the specimen records your observations.

For **Oil Immersion lens (100 X)** for stained/fixed specimens.

a) Place one drop of oil on the slide having a (stained/ fixed) specimen, and place theslide on the stage.

b) Gently turn the revolving nosepiece to oil immersion lens, see that it clicks intoposition.

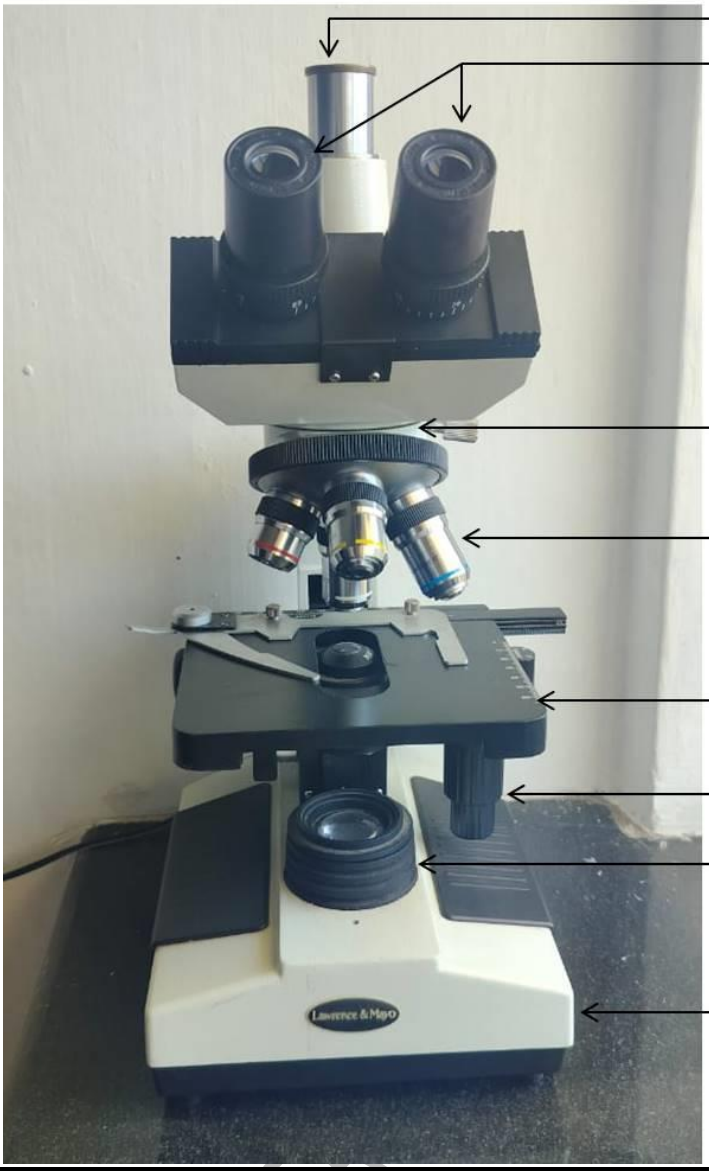
c) Looking from the side raise the stage till the tip of the objective lens just dips into the oil drop. Or use the coarse adjustment knob to lower the objective such that the

lens touches the oil drop.

d) Look through the eyepiece and move the fine focus such that the objective lensslowly moves away from the slide (but should not leave the oil drop) until the image comes into focus.
e) Re adjust the condenser and light intensity if needed.
f) When you see a clear image of the specimen record your observations.

4) Clean and put away the microscope

a) When finished, switch off the light source, lower the stage and remove the slide.
b) Clean the oil immersion lens with a lens cleaning cloth (dipped in xylene).
c) Wipe the stage clean and rotate the nose piece such that the low power objective clicks back into position.
d) Replace the cover of the microscope and carefully put it back in the cabinet.


Trinocular Port
Eye piece
Nose Piece
Objective Lenses
Stage
Stage Control Knobs
Light Source
Base

SOP 2: BACTERIOLOGICAL INCUBATOR

Utility: maintains the accurate temperature for Microbial growth.

Standard Operating Procedure

1. Ensure that the incubator is properly connected to the power supply.
2. Put the main switch on
3. Put the fan on (it ensures the heat is uniformly distributed)
4. Set the temperature to the desired by pressing the SET button
5. To set the temperature desired: Press SET gives value on PV
6. SV gives SP1 (SET pt 1)
7. To change press AT
8. To reset press AT → helps change digit (use the arrow keys for up or down as desired).
9. Use the side arrow key to go from the decimal to unit or tenth place
10. Lock the temperature by pressing SET
11. Record the temperature twice daily i.e. in the morning and in the evening. The temperature should not differ ± 2° C from the set

Care to be taken

1. Switch off the incubator when it is not in use.
2. Do not overload.
3. Incase of any spillage make sure the incubator is disconnected from the power supply and disinfect entirely i.e walls, racks, door, floor etc with 70% ethanol.
4. Reconnect the incubator, set the desired temperature and then replace the contents of the incubator.

Procedure for disinfection

1. Every 6 months make sure the incubator is disinfected in a similar manner
2. The external surfaces have to be regularly dusted

CALIBRATION

1. Set the incubator temperature to the desired
2. Take a calibrated thermometer and dip it in a 500 ml beaker filled to 3/4 of the volume with Glycerol AR grade.
3. Keep the beaker inside, at the centre of the incubator and close the incubator door.
4. Allow the temperature to equilibrate for 30 minutes.
5. Observe the temperature shown by the thermometer.
6. The display and the thermometer should not differ by more than 0.5°C.
7. Any discrepancy has to be reported.
8. The incubator has to be calibrated every month.

1. Keep the incubator in normal vertical position.
2. Insert the trays
3. Load the articles in the incubator keeping proper distance between the articles and the walls of the incubator.
4. Insert the thermometer through the thermometer pocket provided on the top (ensure the thermometer bulb is about 1inch inside the working chamber).
5. Keep the ventilator open about 3-4 mm.
6. Connect the 3 min plug to the mains and put the switch ON.

Setting of Temperature

1. Rotate the knob clockwise for increasing temperature and anticlockwise for decreasing the temperature.
2. Red lamp will be ON and if not, rotate the thermostat knob clockwise so that the red lamp turns ON.
3. When the heaters are ON the temperature of the incubator will now rise and will be indicated by the thermometer.
4. Let the temperature rise to few degrees below the desired temperature.
5. As soon as the above temperature is reached rotate the thermostat knob anticlockwise slowly, and keep it in the same position.
6. When the red lamp turns OFF. The heaters will be OFF.
7. If the red lamp is OFF before reaching the desired temperature rotate the thermostat knob clockwise slightly considering the temperature shown in the thermometer. Likewise repeat this procedure till the above temperature (i.e.) a few degrees below the desired temperature is reached and the red lamp and heaters are OFF.
8. Though the heaters are OFF the temperature will rise a few degrees because of the residual heat, after some time it will start falling automatically (due to heat loss). The thermostat control will turn the incubator ON automatically. Now the temperature will start rising after falling slightly, and then the thermostat control will turn the incubator OFF automatically.
9. Wait for some time and now the thermostat will try to maintain the incubator temperature.
10. The temperature could be set and controlled within ± 0.5° C from 5° C above ambient to max 60° C.

Thermostat knob

Red lamp

SOP 3: COOLING CENTRIFUGE

Utility: To enable separation based on density and size

Calibration

Use of Digital Lab thermometer and Digital Strobe

Check the Instrument temperature displayed and indicator temperature. Check that the overall deviation is within specified accuracy.

Check the Instrument speed displayed and speed reading on Strobe. Check that the overall deviation is within specified accuracy.

Standard Operating Procedure

1. Connect the mains power cord and switch on the mains and then the MCB which is provided on the right-hand side of the unit.
2. The buzzer glows and the display show the last run program parameters
3. Open the lid of the centrifuge using the lid opening lever on the left side of the panel.
4. Attach the appropriate head on the shaft of the motor and tighten the nut firmly with the spanner provided with the centrifuge
5. Insert the appropriate tubes in the head.
6. Balance the head.
7. Close the lid of the centrifuge so/and ensure it locks.
8. After setting all the parameters properly press START. The RUN indicator will light up after a brief period of Automatic Rotor Identification indicated by Ari in the speed display window.
9. The rpm will start increasing slowly depending on the increment value set which will be displayed on the rpm indicator
10. Centrifugation can be stopped at any time by pressing STOP whereby the centrifuge goes into run down mode.
11. When the RUN indicator is gone off / goes off then the door can be opened
12. After the centrifuge has completely stopped moving, remove samples from the centrifuge.
13. Wipe the inside of the centrifuge with a clean cloth.
14. Switch off the centrifuge.
15. In case of malfunctioning of centrifuge press the RESET switch at the rear of unit.

CARE

1. Inspect the centrifuge to ensure it is working properly, there is no damage, and that the centrifuge is able to move freely.
2. Select the appropriate centrifuge tubes inspect them to make sure there are no cracks. Discard any tubes that are cracked or flawed.
3. Never overfill or under fill the centrifuge tubes (follow the manufacturer's limits).
4. Do not balance purely based on volume. This is especially true for solutions with different samples or different concentrations of the same sample.
5. Never use the rotor's maximum run speed.
6. Do not leave the centrifuge until it is operating at full speed and the machine seems to be running smoothly.
7. Ensure that there is no abnormal noise or vibration.
8. NEVER TRY TO HASTEN THE STOPPING PROCESS! Stopping the machine prematurely can lead to injury and mechanical failure.

TROUBLESHOOTING

- ✓ If you notice an unusual noise or vigorous shaking, immediately turn the centrifuge off and remove it from the power source.
    - Typically this is caused by the centrifuge not being balanced properly.
    - Once the machine is at rest, remove the tubes and rebalance.
    - If the problem does not resolve and the centrifuge is still making an unusual noise, it is most likely that the centrifuge is broken, call the repair technician do not use the centrifuge until it is fixed.
- ✓ If the centrifuge is not working.
    - Check that the lid is properly closed and both the latches have engaged.
- ✓ If the centrifuge is not cool
    - Switch on the machine in advance to allow the rotor to be cooled
    - If the hum of the compressor is not detected, call the repair technician and get it fixed.


Stabilizer
On/ Off Switch
Control panel
Lid open/close Catch
REMI
C-24 PLUS
REMI SERVO CONTROLLED VOLTAGE STABILIZER

SOP 4: MICROPIPETTES

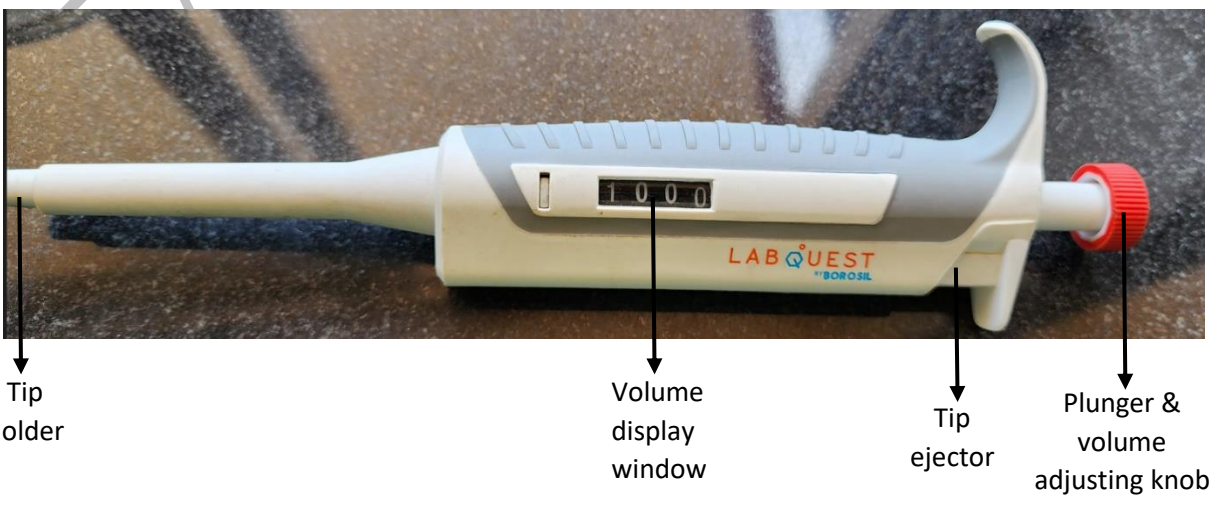
Utility: To dispense liquids of desired volumes

Standard Operating Procedure

1. Select the volume
2. Set the tip
3. Press and hold the plunger at the first stop
4. Place the tip in the liquid
5. Slowly release the plunger
6. Pause for a second and then move the tip
7. Insert the tip into the delivery vessel
8. Press the plunger to the second stop
9. Pause for 2 seconds
10. Remove the tip
11. Release the plunger
12. Eject the tip into the waste container

Care to be taken:

1. Treat micropipettes very gently as they are precision instruments.
2. Keep upright when in use to prevent liquids running inside the shaft of the pipette.
3. Do not leave pipettes lying on the workbench where they can be knocked off and damaged. Before use, make sure the volume has been correctly set.
4. After use, set the pipettes to their maximum capacity.



SOP 5: ANALYTICAL BALANCE

Utility: To weigh compounds accurately.

Standard Operating Procedure

1. Switch on the main power supply of the balance and then press the "On/Off" key on the balance to turn it On.
2. Ensure that the analytical balance is set to the proper units grams (g) or milligrams (mg)
3. Open the sliding door of the balance and place a container or butter paper on the weighing pan.
4. Close the sliding door.
5. Tare the container/butter paper (Press 'T'). The readout will read zero with the container sitting onthe pan.
6. Open the door and add the sample to be weighed on the container or butter paper using a spatula.
7. Close the door, and check for the weight displayed.
8. Accordingly add/remove small quantities of the sample, till the required weight is displayed.
9. Open the door and slowly remove the container or the butter paper containing the weighed sample.
10. Close the door; press the "On/Off" key on the balance and then Switch Off the main power supply to Switch Off the balance.

Care to be taken:

- Switch off the analytical balance when it is not in use.
- Use a moistened soft cloth or tissue paper to clean the weighing pan.
- Use a small artist's brush to remove any residues from the weighing chamber.

SOP 6: COLORIMETER (EQUIPTRONICS (EQ – 651)

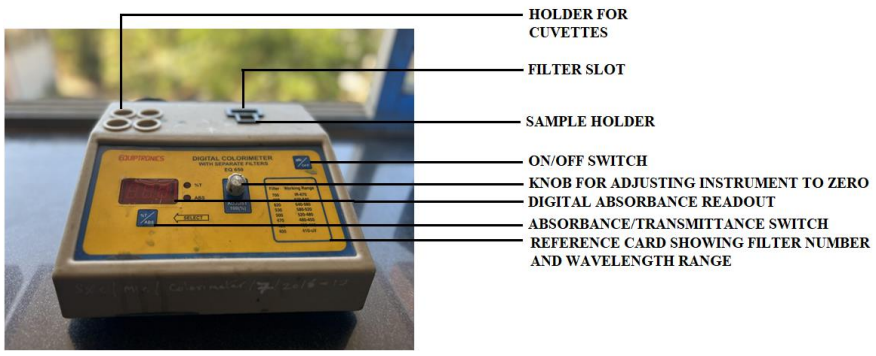
Utility: To record the absorbance

Standard Operating Procedure

1. Insert the mains cable in 230V A.C. outlet.
2. Switch on the instrument at least 5 minutes before use to allow it to stabilize.
3. Insert the desired colour filter in the slot provided. Press it firmly in place. Wait for 1 – 2 minutes.
4. Select the Absorbance/Transmittance on the instrument.
5. Insert cuvette containing blank solution and adjust the absorbance to read zero or transmittance to 100 on the display. Make sure the clear faces of the cuvette are in the light path.
6. If the absorbance is "over range" (usually > 2.0) then the sample must be diluted to yield
    a. a value within the limits of the instrument.
7. Replace the cuvette containing the blank solution with a cuvette containing the unknown solution and note down the reading.
8. Do not disturb Zero and Blank controls while measuring the standard/sample solution.

PRECAUTIONS:

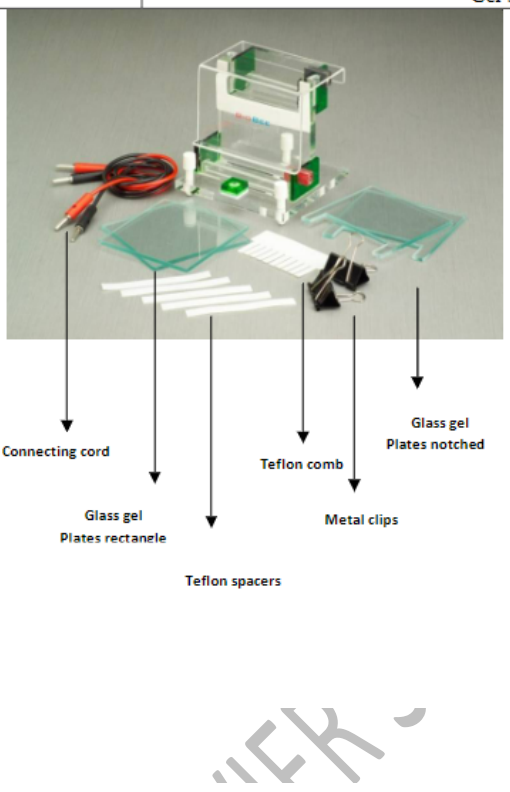
1. The machine must be re-calibrated if a new filter is chosen.
2. The sample cuvettes must be at least two-thirds full.
3. The outside of the cuvettes must be dry and clean.



SOP 7: VERTICAL GEL ELECTROPHORESIS UNIT (POLYACRYLAMIDE GEL ELECTROPHORESIS)

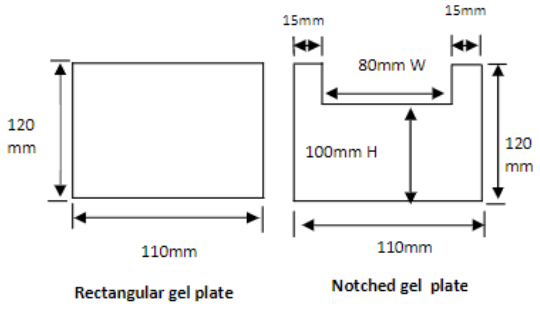
Technical Specifications: BIOBEE® PROTEIN TEACHER consists of: SDS PAGE system tank, 2- sets of glass plates notched and rectangular, 5- well Teflon comb 1.0mm 2-Nos and 1.00m spacers 6 Nos, DC power supply 50/ 100Volded banana cord.

	Description
	BIOBEE™ Vertical Gel (PAGE) Electrophoresis system- Mini, Gel size: 80 X 100 mm (W X H)



Accessories Included:	
Principle material	Imported Acrylic
Glass gel plates	Notched and rectangular 2 Sets
Teflon combs	10-well- 0.5,1 and 1.5mm 1 No each
Teflon Spacers	0.5,1 and 1.5mm 6 No each
Metal Clips	6 Nos
Acrylic clamp and screws	1 Set
Platinum electrodes	1Set (red and black)
Acrylic Boat	1 No
Casting Clamp	3 Nos with nylon screws
Connecting cord	1 No

Glass plate size and gel running Distance



Reagents Required

Acrylamide/bis-acrylamide solution, Gel casting buffers, loading buffer, Tank/Running buffer, 10% SDS, 10% Ammonium persulphate, TEMED.

Standard Operating Procedure

A. Gel casting

1. Clean the glass plates and spacers of the gel casting unit with deionized water and mild detergent.

2. Assemble the plates with the spacers on a stable, even surface.
3. Take a set of rectangular and notched glass plates together.
4. Place a pair of spacers between them at the sides.
5. Seal all 3 sides except notched side with adhesive tape or agarose gel to make glass sandwich water tight for casting gel.
6. Prepare separating gel solution depending on the percentage of gel required and pour the gel solution in the plates assembled with spacers. To maintain an even and horizontal resolving gel surface, overlay the surface with water or isopropanol/butanol.
7. Allow the gel to set for about 20-30 min at room temperature.
8. Prepare stacking gel solution.
9. Discard the overlayed water or isopropanol/butanol on the separating gel.
10. Add the stacking gel solution until it overflows. Insert the comb immediately ensuring no air bubbles are trapped in the gel or near the wells.
11. Allow the gel to set for about 20-30 min at room temperature.
12. Once gel is set, carefully remove the comb.

B. Sample Preparation and Loading

1. To a volume of protein sample, add appropriate volume of loading buffer and loading dye.
2. Protein markers are usually loaded in in one of the end lanes to indicate size of bands.
3. Transfer glass plates (containing cast gel sandwich) into the main tankin the correct orientation.
4. Pour upper and lower tank buffer and switch on current before loading the sample for about an hour to stabilize the flow of ions.
5. Load appropriate volumes of protein sample/marker by inserting the tip of micropipette into the well taking care not to damage wells or induce air bubbles.

3. Gel Running

1. Place the lid and connect to a power supply using banana cord, red to red (positive to positive) and black to black (positive to positive).

2. Adjust to the required voltage/current settings and run the gel (usually 90-225V, 20-45mA).
3. The bubbles or current reading in power supply indicates that the experiment is running.
4. After completion of electrophoretic run, switch off the power (when the loading dye reaches the bottom of the gel).
5. Unscrew the glass plates and gently pry them apart. The gel usually sticks to one of the plates; remove by soaking in buffer and gently lifting with a spatula.
6. Place the gel in staining solution (usually Coomassie or Silver stain) for appropriate time followed by detaining to visualise bands.

Caution:

1. Clean all acrylic systems with warm water and a mild detergent. Do not use ethanol or other organic solvents as they cause it to crack.
2. Acrylamide and bis-acrylamide are neurotoxic in nature. All the steps should be performed wearing powder-free gloves.
3. Casting gels can present hazards from chemical exposure and burns when heating.
4. Running gels presents hazards of electrical shock.

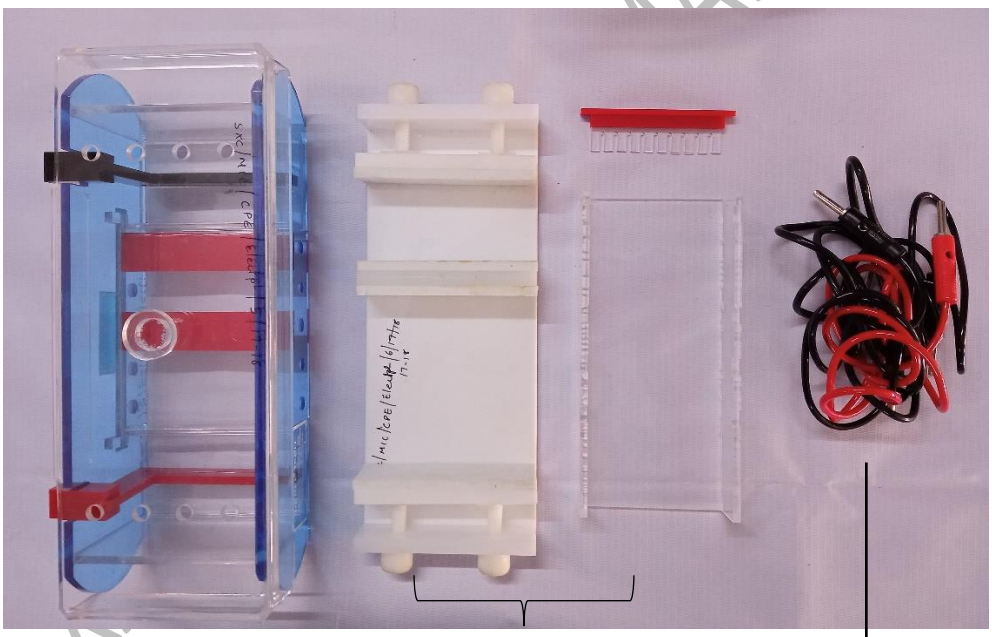
SOP 8: HORIZONTAL GEL ELECTROPHORESIS UNIT

(AGAROSE GEL ELECTROPHORESIS)

Utility: Separates DNA or RNA fragments by size and charge.

Agarose gel electrophoresis is a technique in molecular biology used to resolve nucleic acids (DNA and RNA) and to estimate their molecular weight. DNA/RNA molecules are negatively charged due to their phosphate backbone. During electrophoresis they migrate toward the positively charged electrode. The pores in the gel act as sieves and allow small DNA fragments migrate more rapidly in the gel matrix compared to large fragments. As a result, DNA molecules are separated based on their size.

Technical Specifications:



Gel running tank with positive and negative platinum electrode

Gel casting tray with 8 well comb, white gasket and adjustable nylon

Electrical cords to connect to power supply

Reagents Required: Agarose powder, Gel casting buffers (1X Tris acetate EDTA or Tris borate EDTA), Tank/Running buffer (1X Tris-EDTA Buffer), Loading buffer containing Glycerol, 8X TAE buffer and 0.25% Bromothymol blue, UV Transilluminator

Standard Operating Procedure

1. **Preparation of the gel**
   - Dissolve the appropriate mass of agarose in 1X TAE/TBE buffer using an Erlenmeyer flask. Boil till agarose dissolves completely and a clear solution is obtained. Agarose gels are prepared using a w/v percentage solution. The concentration of agarose in a gel will depend on the sizes of the DNA fragments to be separated, with gels ranging between 0.5%-2%.

     Gel will be hot. Handle with gloves

   - Add 0.5 µg/mL Ethidium bromide to the molten agarose

Ethidium bromide is a suspected mutagen and carcinogen so must be handled cautiously and only using nitrile gloves. It is a hazardous waste and hence must be disposed of according to strict local and/or state guidelines.

2. **Casting of gel**
   - Clean the gel casting unit with isopropanol
   - Assemble the casting tray on a level stable surface and seal both open ends with the gasket to ensure that no gel leaks out.
   - Insert the appropriate combs in order to make wells in the gel.
   - Mix and pour the agarose solution into a gel casting tray which is fitted with the comb. The thickness of the gel should be around 0.5-0.9 cm. Care should be taken to avoid air bubbles.
   - Once solidified, place the gel casting tray into the electrophoretic chamber and pour 1X TAE buffer into the tank till the buffer level stands at 1 cm above the gel surface.Gently lift the comb ensuring that the wells remain intact.

3. **Sample preparation and loading**
    - Take 5µL of loading buffer in a clean eppendorf tube.
    - Add 5 µL of sample DNA or RNA and mix well
    - Carefully load the sample into the well of the gel ensuring that no sample flows out from the well.
    - Molecular weight ladders are usually loaded in one of the end lanes to indicate size of bands

4. **Running the gel**
    - Set up the gel running tank by correctly inserting the positive and negative electrode

      Make sure red wires connect to red port on box and black wires to black port

    - Connect the wires to the correct terminals on the power supply. Plug in the power supply.
    - Cover the electrophoretic tank and switch on the current and run electrophoresis at 50 V for approximately 1 hour.
    - Ensure bubbles start to form on both sides of the gel box at the electrodes.
    - After 45 minutes – 1 hour check that the tracking dye has migrated to atleast 3/4th of the gel.
    - Once the run is completed, turn off power supply and disconnectfrom outlet.

5. **Visualizing the gel**
    - In order to visualize DNA/ RNA bands remove the gel from the casting tray and carefully transfer it to a UV transilluminator chamber.
    - Switch on the UV transilluminator and observe fluorescent orange bands of the DNA/ RNA sample.

Overexposure to UV radiation can cause some forms of skin cancer and eye damage. Proper protective shielding should be used whenever UV light sources are in use.

SOP 9: pH METER

Utility

The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for Operation and Calibration of pH Meter.

Standard Operating Procedure

Make: HANNA, **Model:**pHep **ID No:** HI98107

- **Precautions:**

1. Ensure that the instrument is cleaned before use. If not, clean it. Clean the instrument after use.
2. Check the instrument is kept on the vibration-free table/platform.

- **Basic operation:**

1. Switch on the pH meter by turning the switch in an ON mode.
2. Check the LCD Display status after power on- main screen.
3. The instrument is now ready for use.

- **CALIBRATION:**

1) One- or Two-point calibration with pH 7.01

a) One point

-Dip the tip of the electrode (bottom 4 cms / 1.5 inch) in a sample of pH 7.01 buffer at room temperature. Allow time for the reading to stabilize.

Note: Use a small screwdriver to adjust the pH 7 trimmer on the side of the instrument until the display reads “7.0”. If pH 7.01 is the only calibration point the calibration is finished.

b) Two point

Rinse the electrode with water and dip it in a sample of pH 4.01 buffer solution for acidic samples (pH <7) or pH 10.01 buffer solution for alkaline samples (pH >7). Allow the reading to stabilize.

Note: Use a small screwdriver to adjust the pH 4/10 trimmer on the side of the instrument until the display reads the chosen buffer value.

2) One point calibration with pH 4.01 or pH 10.01

1. Dip the tip of the electrode (bottom 4 cms / 1.5 inch) in a sample of pH 4.01 or pH 10.01 buffer at room temperature. Allow time for the reading to stabilize.

2. If pH 4.01 or pH 10.01 buffer solution is used as the first point the value of the buffer is displayed.

   Note: Use a small screwdriver to adjust the pH 4/10 trimmer on the side of the instrument until the display reads the chosen buffer value.

- **pH measurements**

  1. Calibrate the instrument prior to use as per instructions given above.
  2. Dip the electrode in the desired sample and observe the value on the display.
  3. Rinse the electrode with distilled water after every use.
  4. Wipe the surface dry with a cloth and place the protective cover on the electrode.

ON/OFF Button

Battery compartment

Display


Calibration trimmer (Back side)

Maximum immersion level

pH probe

SOP10: LAMINAR AIR FLOW CABINET

Utility: The Laminar airflow cabinet/hood is equipment that is very commonly used in a microbiology laboratory. The main purpose of it is to provide a contamination-free work environment.

Standard Operating Procedure

1. Ensure that the cabinet is properly connected to the power supply, and switch on the main switch.
2. There are 3 switches on the panel of the instrument for airflow, UV light, and tube light (visible light). Put on the tube light and clean the working area of the instrument with a clean, non-fibre shedding cotton swab soaked in disinfectant (Dettol or 70% isopropyl alcohol)
3. Put off the tube light and switch on the airflow and UV light for 15 minutes before beginning work.
4. Switch off the UV light. Keep on the tube light and airflow throughout the duration of work.
5. On completion of work, clean the working area of the instrument with a clean, non-fibre shedding cotton swab soaked in disinfectant (Dettol or 70% isopropyl alcohol)
6. Turn off the airflow and tube light, and switch off the mains.

Fumigation

1. Clean the working area of the instrument with a clean, non-fibre shedding cotton swab soaked in disinfectant (Dettol or 70% isopropyl alcohol)
2. Place a glass Petri plate containing 20% formaldehyde solution in the centre of the laminar airflow cabinet.
3. Seal the cabinet and allow the formaldehyde to evaporate for 2 hours.
4. To neutralise the formaldehyde vapours, replace the Petri plate containing formaldehyde with 25% ammonium solution or 25% ammonium hydroxide solution and allow it to evaporate for 3 hours.

Care to be taken

1. Switch off the laminar airflow when not in use
2. No operations must be carried out when the UV light is switched on

3. The operator must be dressed in lab coats and gloves while working in the laminar airflow cabinet
4. Formaldehyde is highly toxic. Care should be taken that the laminar airflow cabinet and the room harbouring it must be completely sealed during the fumigation process.

SOP 11: HOT AIR OVEN

Utility: Used for drying of glassware and sterilisation of chemicals not suitable for moist heat sterilisation.

Standard Operating Procedure

1. Ensure that the hot air oven is properly connected to the power supply, and switch on the main switch.
2. Load the glassware to be dried on the shelves of the oven, ensuring that no items touch the edges.
3. When switched on, the green light at the base of the instrument glows.
4. Ensure the door of the oven is closed tightly and allow temperature to build to 200 ° C for 2 hrs.
5. On completion of drying, switch off the instrument and allow to cool.
6. Empty contents of the oven.

Care to be taken

1. Switch off the hot air oven when not in use
2. No operations must be carried out when the oven light is switched on

Thermostat

SOP 12: AUTOCLAVE

Utility: For moist sterilisation of media, glassware and for decontamination of used agar, cultures.

Precautions:

a. Ensure that the material is autoclavable. (Oils, waxes, some plastics, flammable materials, and samples containing solvents or substances that may emit toxic fumes should not be autoclaved)

b. Package material suitably.

c. Bottles with liquids must be a maximum of one-half full and lids loosened.

d. Contaminated materials must be secured in a containment vessel or an autoclavable bag and processed in a different autoclave

e. Ensure there is sufficient water in the base of the autoclave.

Standard Operating Procedure

Loading the Autoclave

a. Wear heat insulating gloves, eye protection, lab coat, and closed-toed shoes

b. Place items in basket container, then place into the autoclave chamber

c. Ensure there is enough room in the autoclave for steam to circulate.

The bottom rack must always be used to allow steam to circulate underneath the secondary container.

Operation of the Autoclave

1. Open the lid by unscrewing fly nuts.
2. Pour distilled water in the chamber upto the top level of the heater cover stand kept at the bottom.
3. Load the material to be sterilized in the carriers.
4. Close the lid and tighten all the fly nuts (opposite ends simultaneously) with equal pressure to ensure uniform pressure on rubber gasket.
5. The pressure will now steadily rise and stabilize between 15 and 16.5 psi.
6. The safety valve is purposely set above 15 psi in order to avoid under sterilization.
7. The materials are kept for 20 mins or as required.

8. After the holding time is over, switch off the mains switch or MCB as the case may be, remove all the steam through the exhaust valve, before opening the fly nuts and make sure the fly nuts are not prematurely open /loosened even at 1-2 psi.
9. Unload the material.