DEPARTMENT OF BIOCHEMISTRY

STANDARD OPERATING PROCEDURE

for the

EQUIPMENTS

DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology
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DEPARTMENT OF BIOTECHNOLOGY

MINISTRY OF SCIENCE AND TECHNOLOGY, MHRD,
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DIGITALBALANCE

DIGITALBALANCE

Electronic balance is a significant instrument for the laboratories for **precise measurement of chemicals** which are used in various experiments.

PROTOCOL

- Insert plug in socket.
- Switch on the instrument at least 10 minutes before use.
- Adjust to zero.
- Place the weighing paper on the pan and use tare to reset zero.
- Weigh the required quantity of sample.
- Remove the paper along with sample weighed.
- Adjust to zero.
- Switch off and clean the balance.
- Make an entry in the log book.

DIGITALCOLORIMETER

DIGITALCOLORIMETER

Colorimeter is a device used to measure the wavelengths of light that helps to find the concentration of a particular unknown substance.

PROTOCOL

- Insert plug in the socket.
- Switch on the instrument at least 15 minutes before use.
- Ensure to keep water blank before switching on the instrument.
- Set the required wavelength.
- Select % T using the knob and adjust to 100%.
- Switch the knob to O.D which should be zero.
- Clean the cuvettes after use.
- Make an entry in the log book.

UV VISIBLE SPECTROPHOTOMETER

UV–Visible spectrophotometer is used to study the optical absorbance of the thin/thick films or any chemical substance or light absorbance of the systems.

PROTOCOL

Before operation ensure that the instrument is clean and calibrated.

- Turn ON the main power of the instrument and computer connected with the instrument.
- After turning the power ON spectrophotometer will start the initialization to check the functions of all parameters. Initialization will be completed after 5 minutes.
- Wait for 15 minutes for the proper stabilization and illumination of UV lamp before operation.
- Do not interrupt the initialization procedure, it is important as it performs the checks for functioning of all instrumental parameters each parameter will be highlighted which functions normally as the initialization proceeds and if any parameters is not functioning normally and the initialization will stop automatically.
- Spectrophotometer can also be operated by its function keys provided on the instrument which displays their response on the LCD screen of the instrument.
- UV Probe software is commonly installed to operate the UV VIS spectrophotometer.
- To run the software double click on the UV Probe software icon displayed on the desktop of the computer. Login the main window by entering the individual login ID and password.
- The two modes of measurements were: Photometric mode and Spectrum mode

  PHOTOMETRIC MODE: It is used to measure the absorbance transmittance (T%) at affixed wave length.

- To connect spectrophotometer with software click on connect after connecting a green color indicator will be highlighted on the computer

  Select [Window] → [Photometric], it will display UV Probe (system Administrator)-

  [Photometric].

- Create the method enter the wavelength & add the parameter, select calibration and enter raw data and save the method.
- For blank correction, take two clean cuvettes, after proper rinsing keep the blank solution prior to the measurement in both cuvette and wipe them clean from the outside using tissue paper.
- Place one cuvette in the reference cell holder and the other in the sample cell holder. Close the sample compartment.
- Press 'AUTO ZERO' key. In case of multi wavelength press 'Baseline key.
- After Blank correction, keep the sample in the sample compartment. Click on 'Read Unk.' to measure the absorbance.
- After completion of measurement remove the cuvettes and wash them with purified water followed by acetone, so that the cuvettes get dried easily.
- Click Report button, it will display report template of respective test.
- Press the "PRINT" key to take the result print.

SPECTRUM MODE: Used to measure the absorbance of sample in wide range of selected wavelength

it will scan the sample in selected range and produces the spectra.

Select [Window] → [Spectrum], it will display UV Probe (system Administrator) – [Spectrum].

- To create the measurement file select method enter the wavelength range from start to end select scanning rate at medium speed then save the method file at desired path (If method is already exist then open the method and do the baseline correction and scan the sample).
- For baseline correction take two clean cuvettes, After proper rinsing, keep the blank solution prior to the measurement in the both cuvettes and wipe them clean from the outside using tissue paper.
- Place the cuvette in the sample cell holder. Close the sample compartment.
- Click on baseline button, the "Baseline Parameters" file gets opened, select the wavelength range (nm) start to end. And click on 'OK' to measure the baseline.
- Keep the standard or sample in sample compartment. Click on Start button for scanning, it will display the [New Data Set] , Save the data with respective name and path.
- After scanning, Select [Operation] → [Peak Pick] to the data at each wavelength of the spectrum can be read.
- Select Report button, it will display report template of respective test.
- Press the "PRINT" key to take the result print.

AGAROSE GEL ELECTROPHORESIS

Agarose gel electrophoresis is widely used for separation of DNA and RNA samples in events like restriction fragment analysis, polymerase chain reaction product analysis, checking the integrity of genomic DNA, and purification of nucleic acids.

PROTOCOL

PREPARATION OF THE GEL

- Weigh out the appropriate mass of agarose into an Erlenmeyer flask. 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 most gels ranging between 0.5%-2%. The volume of the buffer should not be greater than 1/3 of the capacity of the flask.
- Add running buffer to the agarose-containing flask. Swirl to mix. The most common gel running buffers are TAE (40 mM Tris-acetate, 1 mM EDTA) and TBE (45 mM Tris-borate, 1 mM EDTA).
- Melt the agarose/buffer mixture. This is most commonly done by heating in a microwave, but can also be done over a Bunsen flame. At 30 s intervals, remove the flask and swirl the contents to mix well. Repeat until the agarose has completely dissolved.
- Add ethidium bromide (EtBr) to a concentration of 0.5 µg/ml. Alternatively, the gel may also be stained after electrophoresis in running buffer containing 0.5 µg/ml EtBr for 15-30 min, followed by destaining in running buffer for an equal length of time.
- NOTE: EtBr is a suspected carcinogen and must be properly disposed as per institution regulations. Gloves should always be worn when handling gels containing EtBr. Alternative dyes for the staining of DNA are available; however EtBr remains the most popular one due to its sensitivity and cost.

- Allow the agarose to cool either on the benchtop or by incubation in at 65 °C water bath. Failure to do so will warp the gel tray.
- Place the gel tray into the casting apparatus. Alternatively, one may also tape the open edges of a gel tray to create a mold. Place an appropriate comb into the gel mold to create the wells.
- Pour the molten agarose into the gel mold. Allow the agarose to set at room temperature. Remove the comb and place the gel in the gel box. Alternatively, the gel can also be wrapped in plastic wrap and stored at 4 °C until use

2. SETTING UP OF GEL APPARATUS AND SEPARATION OF DNA FRAGMENTS

- Add loading dye to the DNA samples which are to be separated. Gel loading dye is typically made at 6X concentration (0.25% bromphenol blue, 0.25% xylene cyanol, 30% glycerol). Loading dye helps to track how far your DNA sample has travelled, and also allows the sample to sink into the gel.
- Program the power supply to desired voltage (1-5V/cm between electrodes).
- Add enough running buffer to cover the surface of the gel. It is important to use the same running buffer as the one used to prepare the gel.
- Attach the leads of the gel box to the power supply. Turn on the power supply and verify that both gel box and power supply are working.
- Remove the lid. Slowly and carefully load the DNA sample(s) into the gel. An appropriate DNA size marker should always be loaded along with experimental samples.
- Replace the lid to the gel box. The cathode (black leads) should be closer the wells than the anode (red leads). Double check that the electrodes are plugged into the correct slots in the power supply.
- Turn on the power. Run the gel until the dye has migrated to an appropriate distance.

3. OBSERVING SEPARATED DNA FRAGMENTS

- When electrophoresis has completed, turn off the power supply and remove the lid of the gel box.
- Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.
- Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system. DNA bands should show up as orange fluorescent bands. Take a picture of the gel
- Nucleic acids running on an electrophoresis can be detected by staining with a dye and visualized under 300-nm UV light. Staining and visualization of DNA are conducted by using either ethidium bromide or SYBR Green. The most convenient and commonly used method to visualize DNA in agarose gel is ethidium bromide.
- The banding pattern of DNA resolved through the gel by recorded images. Images of ethidium bromide stained gels may be captured by using transmitted or incident UV light.
- Properly dispose of the gel and running buffer as per institution regulations.

SDS PAGE ELECTROPHORESIS

SDS PAGE or Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis is used for the separation of proteins based on their molecular weight. It is a technique widely used in forensics, genetics, biotechnology and molecular biology to separate the protein molecules based on their electrophoretic mobility.

PROTOCOL

- Decide which percentage of gel you need to separate your proteins
- Eg. 1: Use 4–8% gels to separate proteins 100–500 kDa in size.
- Eg. 2: Use 4–20% gels to separate proteins 10–200 kDa in size.
- Place your gel in a clean plastic electrophoresis chamber and corresponding gel holder.
- Prepare 1X SDS-PAGE Running Buffer as follows: for 500 mL of 1X SDS-PAGE Running Buffer by adding 50 mL of 10X SDS-PAGE Running Buffer (MB-017) to 450 mL of diH_20 (MB-009-1000).
- Fill the inner portion between the gel(s) and the gel holder with the appropriate 1X Running Buffer Sample Preparation:

- If using a pre-prepared lysate (already in sample buffer), thaw lysate and transfer 25 µL of lysate to a clean pre-labeled microcentrifuge tube. Add β-mercapto ethanol (BME) to a final concentration of 0.55M, i.e. add 1 µL stock BME per 25 µL lysate. Mix well by pipetting. Label microcentrifuge tubes with sample description, volume and concentration of lysate.
- Any other protein samples: transfer to clean pre-labeled microcentrifuge tubes and mixed with an equal volume of 2X Sample Buffer (MB-018) with 0.55M BME. Sample protein concentration should be sufficiently high; eg.: final protein concentrations from 1 µg–500 µg depending on protein type and detection method.
- Prepare MW standards (MB-201-0200) for electrophoresis. For SDS-PAGE use either an unstained MW standards or pre-stained MW marker. For SDS-PAGE followed by western blotting, use pre-stained MW markers.
- Record lane number, sample description, sample concentration, loading volume, loading amount and addition of reducing agent for all samples.
- Place all micro centrifuge tubes containing samples for SDS-PAGE into a heating block (set to 95°C) or water bath. Heat samples for 5 minutes.
- After heating, centrifuge the aliquots for 3 minutes using a micro centrifuge to pellet any debris. Pour the remaining 1X Running Buffer into the outer chamber.
- Load all samples into gel lanes starting with the MW standards. Sample loading volumes should be from 5 µL–35 µL per lane (depending on gel). If protein concentrations are from 100 µg/mL–500 µg/mL, then sample amounts will range from 0.5 µg–17.5 µg per lane. **Note:** Generally, 1.0 µg is sufficient to visualize purified proteins and 10 µg is sufficient to visualize proteins in lysates on a coomassie stained gel.
- Cover the chamber and firmly connect both the anode and the cathode. Set the voltage on the electrophoresis power supply to a constant voltage of 150 V. Turn ON the power supply.
- Allow the gel to electrophoresis for 45–90 minutes. Turn OFF the power immediately after the dye front migrates out from the bottom of the gel.
- Disconnect the electrodes and remove the cover. Remove gel holder from the electrophoresis chamber. Carefully remove the gel from holder. Remove the gel from its plates and proceed with desired detection method.

ORBITAL INCUBATOR SHAKER

ORBITAL INCUBATOR SHAKER

Orbital Incubator shaker is used for culturing microbes, washing blots and general mixing.

PROTOCOL

- Turn on the apparatus by power switch. This apparatus is capable of time and temperature planning, up to three intervals and three temporal ranges.
- Press the TEMP button. Set the required temperature by downward and upward arrows then the press square button. When different temperatures are required repeat this process for T1, T2 and T3 three times. If you need the same temperature for a long time adjusts all the mentioned factors on one required temperature.
- Press the TIME button. Set the required time which is relevant to adjust temperature by downward and upward arrows then press the square button. When different times are required repeat this process for Time1, Time2 and Time3 three times. If you need to work with apparatus for a long time adjust all the mentioned factors on one required time.
- Set the apparatus shaking rate by pressing the relevant SHAKE button and press the square button to stabilize it.
- Press the START button then turn on the SHAKE switch. The temperature flasher of the apparatus starts blinking to show the apparatus has been started.
- Press the STOP button after determinate duration then turn off the apparatus using POWER switch.

DIGITAL FLAME PHOTOMETER

DIGITAL FLAME PHOTOMETER

Digital flame photometer is used in inorganic chemical analysis to determine the concentration of certain metal ions such as sodium, potassium, lithium and calcium.

PROTOCOL

- Both the standard stock solution and sample solution are prepared in fresh distilled water.
- The flame of the photometer is calibrated by adjusting the air and gas. Then the flame is allowed to stabilize for about 5 min. Now the instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters.
- The readings of the galvanometer are adjusted to zero by spraying distilled water into the flame.
- The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame. Now the full scale deflection of the galvanometer is recorded.
- Again distilled water is sprayed into the flame to attain constant readings of galvanometer. Then the galvanometer is readjusted to zero.
- Now each of the standard working solutions is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Finally sample solution is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Calculate the mean of the galvanometer reading.
- Plot the graph of concentration against the galvanometer reading to find out the concentration of the element in the sample. The solvent is first aspirated to obtain fine solid particles.
- These molecules in the solid particles are moved towards the flame to produce gaseous atoms and ions.
- These ions absorb the energy from the flame get excited to high energy levels from the ground state.
- But as these ions are unstable, they return back to ground state. While returning they emit characteristic radiation.
- The intensity of emitted light is proportional to the concentration of the element

AUTOCLAVE

AUTOCLAVE

Autoclaves operate at high temperature and pressure in order to kill microorganisms and spores. They are used to decontaminate certain biological waste and sterilize media, instruments and lab ware.

PROTOCOL

LOADING AUTOCLAVE

- Wear heat-insulating gloves, and closed toed shoes. Place material in autoclave. Do not mix incompatible materials.
- Do not overload; leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air. Close and latch door firmly.

OPERATING AUTOCLAVE

- Check to be sure that the water reservoir is filled (approximately 1" from top), and if not, fill with distilled water.
- Choose appropriate cycle (e.g. fluid, dry etc) for the material. Set appropriate temperature for the cycle.
- Turn the autoclave to fill and wait until the water in the bottom of the autoclave covers the fill plate. Turn the autoclave to sterilize and load autoclave. Close and lock door
- A steam cycle is approximately 40 minutes if the autoclave is cold and approximately 20 minutes if it is already warmed from a previous steam cycle.
- When cycle is complete, turn autoclave to **VENT** and remain there until pressure drops. The heat stays on during the venting and without the steam plastics will melt.
- Turn the autoclave to **POWER OFF.** Do not attempt to open the door while autoclave is operating. The manuals for operation of the autoclave are located on the wall behind the autoclave.

UNLOADING AUTOCLAVE

- Wear heat-insulating gloves and closed toed shoes.
- Ensure that the pressure of the chamber is '8' before opening the door.
- Wear gloves and stand back from the door as a precaution, carefully crack door open no more than 1 inch (2.5 cm) to release residual steam and allow pressure within liquids and containers to normalize.
- Allow sterilized material to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.
- Do not agitate containers of super-heated liquids or remove caps before unloading.
- After removal from the autoclave, place hot items in an area which clearly indicates the items are 'hot' until the items cool to room temperature. Push door closed.

LAMINAR AIR FLOW CHAMBER

LAMINAR AIR FLOW CHAMBER

Laminar air flow is used for **aseptic transfer of microbial culture**. It is used for providing an excellent aseptic work bench for processes like inoculations, sub-culturing, sterile drug preparation, assays, etc.

PROTOCOL

- Laminar Air Flow Chamber is used to perform aseptic work.
- Swab the floor of LAF with spirit or 70% alcohol.
- Put on the UV light for 20 minutes (LAF lid should be closed).
- After 20 minutes switch off the UV light and switch on the light and air flow.
- Again swab the floor and side wall of the LAF with spirit.
- Switch on the burner to do the aseptic work.
- After completion of work put off the burner, switch off the airflow and clean the hood, swab it again with spirit or alcohol.
- Close the lid. Switch off the light and airflow.
- Switch on the UV light again for 20 minutes and then switch off.

PLANT TISSUE CULTURE RACK

PLANT TISSUE CULTURE RACK

Plant tissue culture is an in-vitro culture of plant cells, tissues or organs which will form a complete plant. Tissue culture racks are very useful for various types of tissue culture plants and other tissue culture work in tissue culture room

PROTOCOL

- **SELECTION OF PLANT**: The plant which has to be cultured in vitro is selected.
- ISOLATION OF EXPLANT: Explant is any part of plant excised out for tissue culture.
- STERILIZATION OF EXPLANT: Explant is surface sterilized so as to avoid contamination.
- INOCULATION OF EXPLANT: The explant is inoculated on the nutrient medium. For plant tissue culture, the most common medium used is MS media.
- INCUBATION: After inoculation, the cultures are incubated so as to provide proper conditions for their growth and regeneration like temperature, moisture etc.
- INITIATION OF CALLUS: Callus is a mass of undifferentiated cells formed by the dedifferentiation of plant cells or the explant. Callus further regenerates to form roots and shoots and eventually the complete plant.
- SUB-CULTURING: The cultured cells or tissues are transferred regularly to new nutrient medium and this is called subculturing.
- REGENERATION: Regeneration is the formation of organized structures like roots, shoots, flower buds etc from the cultured cells
- HARDENING: The plantlets are removed from the cultures and prepared for soil transfer and this transfer to fields is known as hardening.