DEPARTMENT OF ZOOLOGY

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Standard Operating Procedures (SoP's)

STANDARD OPERATING PROCEDURE FOR AUTOCLAVE

Operating instructions:

1. Unscrew the nuts to access the chamber.
2. Fill the chamber with distilled water upto the top level of the heater covers stand placed at the bottom of the chamber.
3. Load the material to be sterilized in the carriers.
4. Lower the lid on to the gasket. Tighten all the flynuts diagonally two at a time with equal pressure to ensure uniform pressure on the rubber gasket. This will prevent any leaks during the operation.
5. Turn the machine on using the mains switch or the MCB.
6. The vacuum breaker and the purge valve automatically vent the internal state air (purging). You will see steam escaping from the same. Once the chamber is purged of all air, steam pressure will close this valve. This will ensure proper sterilization using steam only.
7. The pressure will now steadily rise and stabilize between 15 and 16.5psi. The valve may start leaking lightly from 12psi onwards, which is normal.
8. The safety valve is purposely set above 15psi in order to avoid under sterilization.
9. Keep the material for 20 minutes or a long as required.
10. After the hold time has elapsed, turn the machine off using the mains switch or the mobs. Remove all the steam through the exhaust valve, before opening ther flynuts. Never prematurely open / loosen the flynuts, even at 1-2psi.
11. Unload the material.
12. Top up the chamber with distilled water, refer to instruments 2

Electric Shock:
In case of water spillage near the electric panel, there is a chance of electric shock.
Pressure:
After completion of sterilization, lid should be opened only if pressure gauge shows 0 pressure. Otherwise, there are chances of burns.
Injury/ Exposure Response Procedure:
First aider should immediately take care of injury, if any. The first aiders should seek appropriate medical help if required.

STANDARD OPERATING PROCEDURE FOR CENTRIFUGE

Procedure:

1. Turn ON the electrical power to the centrifuge.
2. Load the centrifuge tubes into the rotor.
3. Close the lid of the centrifuge.
4. Select the required time by adjusting the timer knob.
5. Select appropriate speed with the speed controller.
6. Wait next to the centrifuge until it has reached the desired speed and has not stopped due to it being imbalanced.
7. Wait until the centrifuge run has being completed.
8. Unscrew the rotor lid and remove the samples.
9. Switch off the instrument after use.

Spill Response Procedure:

1. In case of spillage (water/ solution/ buffers), mop with absorbent.
2. Then clean up with soap solution.
3. Report spillage in the log book.

Precautions:

1. Tubes are to be filed to the sample level with the same density fluid.
2. Ensure the tubes to be centrifuged are matched in pairs or evenly distributed around the rotor.
3. If centrifuge is vibrating a little too much, stop the rotor and wait until it has stopped spinning and redistribute the tubes to make sure the rotor is completely balanced.
4. Entry in log book is mandatory

STANDARD OPERATING PROCEDURE FOR pH METER

Name of the procedure:

Measurement of pH

Operating Instruments:

1. Switch on the instrument.
2. Remove the electrode dipped in electrode buffer (3M KCI)
3. Rinse with distilled water.
4. Dry the outer surfaces of the electrode with a clean dry tissue.
5. Dip electrode in solution whose pH has to be measured.
6. After use rinse the electrode in distilled water, dry and dip the electrode back in the electrode buffer.
7. Switch off the instrument after use.

Protective Equipment:

Lab coat should be worn while using the instrument.

Waste disposal procedure:

All waste should be diluted and disposed in the sink.

Injury/ Exposure Response Procedure:

Steps to be taken in the event of an accident and appropriate first aid should be provided.

Spill Response Procedure:

In case of spillage (water/ solution/ buffers), mop with spill absorbent. Then clean up with soap solution.

Note:

1. *Only the people trained by one of the personnel below are authorized to use the pH meter.*
2. *The electrode should always be immersed in the electrode buffer (Before and after use).*
3. ***DO NOT** rub the membrane of the electrode.*
4. *The instrument should not be used to measure pH of viscous solutions.*
5. *In case the instrument is nonfunctional, seek help from trained personal.*
6. *Entry in log book is mandatory.*

STANDARD OPERATING PROCEDURE FOR PAGE ELECTROPHRESIS

1. Clean the whole PAGE apparatus with ethanol and let it dry.
2. Arrange the apparatus by placing the ceramic plate behind glass plate, separated by specific spacers.
3. Pour 1% molten agarose on the bottom of the gel casting unit to seal the bottom of the glass plates, so that the gel will not leak out.
4. Place the glass plate assembly vertically on the gel casting unit. Tighten it with the help of screws.
5. Pour the gel in the gel casting unit, insert the comb and allow it to set.
6. Transfer the gel in the gel running unit and tighten it with the help of clamps.
7. Fill the tank buffer upto the brim. Then load the sample in the wells prepared by using a comb.
8. Connenct the AC convertr to the gel running unit by connecting Anode to Anode (red) and Cathode to Cathode (black).
9. Switch on the power supply and keep the voltage at 80V.

Hazard:

Monomer of acrylamide is neurotoxic hence it should be handled wearing gloves with care.

Protective Equipment:

Lab coat and gloves should be worn while performing the experiment.

Waste Disposal procedure:

Used plastic tubes or micro-tips should be disposed into the respective bins.

Gel should be wrapped in paper and disposed.

Note:

1. Once the usage is over please switch off the main switch.
2. Entry in log book is mandatory.

STANDARD OPERATING PROCEDURE FOR BALANCE

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

STANDARD OPERATING PROCEDURE FOR COLORIMETER

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

STANDARD OPERATING PROCEDURE FOR WATER BATH

1. Fill the water bath to half its capacity.
2. Turn on the main supply.
3. The red LED bulb will glow.
4. Press SET key, SP will be displayed on the digital panel.
5. Set the required temperature by using the "UP" and "DOWN" keys and press ENTER.
6. Set the required percentage of power to 100 % and press ENTER key twice till the heater is switched on.
7. Once the temperature is attained, the thermostat will maintain the temperature.
8. After usage switch off the main power supply.
9. Empty out the water bath,

Use of Micropipettes for cultures

1. Choose a **fixed volume** micropipette depending on the amount of culture you need to pipette out to perform an experiment.
2. Hold the culture tube in your left hand and open the cotton plug.
3. With your right hand secure a suitable sterile micropipette tip with the micropipette holding it vertically.
4. Now gently push the plunger/piston with your thumb to the first level.
5. Dip the tip in this position into the culture tube to aspirate the required amount by releasing the plunger.
6. Always remember that the tube and the micropipette should be at the eye-level held vertically.
7. Close the culture tube and then unplug another sterile test tube into which you need to transfer the culture.
8. Gently eject the fluid out into the tube and expel the last drop by pushing the plunger to the second level.
9. Discard the tip in a discard container that contains a disinfectant. To do this, eject the tip by pressing the large button at the top of the micropipette.

Precautions

1. Always hold the micropipette vertically when in use to prevent backflow of the culture and fouling of the pipette.
2. Never forget to apply a tip to the micropipette while using it.
3. Gently depress or release the piston to avoid malfunctioning of the same.
4. For **variable pipettes**, set the volume only within the range specified on the pipette. Do not try to go beyond the minimum and maximum ranges to avoid causing any damage to the gears.
5. Always store the pipettes at the specified maximum volume only, to prevent any torsional stress on the gears. **Please note: 1 ml = 1000µL 0.5 ml = 500µL 0.1 ml 100µL**

RULES TO BE FOLLOWED WHEN USING A LIGHT MICROSCOPE

1. The microscope is a part of everyday practical work. It is an instrument of precision and good care must be taken to preserve its accuracy.
2. Remove the microscope from its numbered box with one hand supporting the base and the other holding the limb of the microscope.
3. Place the microscope on the working table with the mirror facing the tube light.
4. Revolve the nose piece and put the low power in alignment. Raise the lowered condenser and open its iris diaphragm. Adjust the mirror for light using either surface, plane or concave so that an image of the light source is obtained in the front lens of the condenser. The concave surface of the mirror gives a larger image of the light source.

5. Look through the eyepiece and check for the microscopic field's brightness. If not sufficiently illuminated, the mirror must be shifted to obtain maximum brightness.
6. Place the specimen on the stage and move the stage knobs to properly centre the slide such that the specimen is immediately below the objective lens and above the front lens of the condenser. Lower the barrel of the microscope to the maximum without looking through the eyepiece. Then looking through the eyepiece raise the objective till the image comes into focus. Use the coarse adjustment. Fine focusing is done by using the fine adjustment.
7. Scan the microscopic fields to view the images of the objects. Record your observations in the form of a labeled diagram and an observation table.
8. After the microscopic work is over, the oil- immersion lens must be cleaned with xylene or xylol on a piece of tissue paper to remove the oil. Use a clean tissue to remove the excess xylol from the oil-immersion lens' surface.
9. Lower the condenser after closing the iris diaphragm.
10. Put the low power in alignment with the eyepiece and lower the barrel of the microscope.
11. Place the microscope in its numbered box.

PREPARATION OF MOLECULAR BIOLOGY STOCK AND WORKING SOLUTIONS

Background:

Stock solution: The solution having a concentration many folds higher than actual experimental requisition. Working solution: The solution with desirable concentration to be used in the experiment. The mathematical equation used for making working solution from the stock solution is: $C1V1 = C2\ V2$ Where C1 = Conc. of the working solution, V1 = Volume of the working solution, C2 = Conc. of the stock solution, V2 = Volume of the stock solution. Material and Reagent Requirements: Volumetric flasks (100 ml), Beakers (100 ml, 200 ml), Reagent bottles (250 ml), Falcon tubes (50 ml), measuring cylinder, Whatman filterpaper, Funnel, Pipettes, Sterile double distilled water, Magnetic stirrer, Hot plate, pH meter, Standard pH buffers (pH: 4,7 & 9), magnetic bead, weighing balance, Aluminum foil, CTAB, NaCl, EDTA, Tris-HCl, Sodium acetate, glacial acetic acid, ammonium chloride, ammonium acetate, SDS, glucose, NaOH, Potassium acetate, Conc. HCl, ethidium bromide, lysozyme, xylene cyanol, Bromophenol blue, sucrose.

Methodology:

A. Stock Solutions 1. Tris-HCl (1M; pH 8.0): Dissolve 12.114g of Tris base in 70ml of sterile double distilled water. Adjust pH to 8.0 with 1N HCl and the final volume is thereafter made 100 ml. 2. Ethylene di-amine tetra acetic acid (EDTA) (0.5M; pH 8.0): Dissolve 18.61g of EDTA in 50ml of sterile double distilled water and pH is adjusted to 8.0 with 10M NaOH solution. The final volume is made 100 ml with sterile double distilled water. 3 3. Sodium Dodecyl Sulphate (SDS) (10%): Dissolve 10g of SDS in 100ml of sterile double distilled water with gentle heating at 65oC. 4. Ammonium acetate (CH3COONH4) (7.5 M): Dissolve 57.8g of CH3COONH4 in 50ml of sterile double distilled water and the final volume is made to 100ml with sterile double

distilled water. 5. Cetyltrimethyl ammonium bromide (CTAB) (10%): Dissolve 10g of CTAB in 100ml of sterile double distilled water with gentle heating at 65◦C. 6. Sodium Chloride (NaCl) (5M): Dissolve 29.2 g of NaCl in 80 ml of sterile double distilled water. Adjust the volume to 100 ml with double distilled water. 7. Sodium acetate (CH3CooNa.3H2O) (3M, pH 5.2): Dissolve 40.8 g of CH3CooNa in 80 ml of sterile double distilled water. Adjust the pH to 5.2 with glacial acetic acid. Make the final volume to 100 ml with double distilled water. 8. Sodium hydroxide (NaOH) (10N): To 80 ml of sterile double distilled water slowly add 40 g of NaOH pellets with continuous stirring. Adjust the final volume to 100 ml with sterile double distilled water. 9. Ammonium Chloride (NH4Cl) (1M): Dissolve 5.35 gm of ammonium chloride in 80 ml of sterile distilled water and final volume of the solution is made to 100 ml. 10.Gel Loading Dye (6x): 0.025 % of bromophenol blue with 40% (w/v) sucrose in water. 11.Ethidium bromide (10 mg/ml): 10 mg of ethidium bromide dissolved in 1 ml of sterile double distilled water and stored in a dark bottle at RT. 12.10x Gel loading dye: 0.5% (w/v) xylene cyanol dissolved with 40% (w/v) sucrose in water. 13. Lysozyme (20mg/ml): Dissolve lysozyme at a concentration of 20mg /ml in 10 µl of 1M Tris, pH 8 and aliquots of 200µl are made and store at -20oC for further use. 4 B. Working Solutions:

1. TE buffer (Tris-HCl, 10 mM, EDTA 1mM; pH 8.0): 1ml of Tris-HCl (1M; pH 8.0) is mixed with 200µl of EDTA (0.5M, pH 8.0) and the final volume is made to 100 ml with sterile double distilled water. 2. RBC Lysis buffer (Tris-HCl,10 mM; EDTA ,1mM; NH4Cl,125mM; pH 8.0): 1ml of Tris-HCl (1M; pH 8.0) is mixed with 200µl of EDTA (0.5M, pH 8.0) and 12.5 ml of NH4Cl (1M). The final volume is made to 100 ml with sterile double distilled water. 3. Plant Genomic DNA Extraction Buffer (CTAB, 2%; NaCl, 1.4 M; EDTA,20 mM, pH 8; Tris-HCl, 100 mM, pH 8): Mix 20 ml of CTAB (10%), 28 ml of NaCl (5M), 10 ml of Tris-HCl (1M; pH 8.0) and 4 ml of EDTA (0.5M, pH 8.0). The final volume is made to 100 ml with sterile double distilled water. 4. TAE buffer (50X): Add 242 g of Tris-HCl base in 500 ml of sterile double distilled water. Subsequently mix 57.1 ml of glacial acetic acid and 100 ml of 0.5 M EDTA (pH 8). The final volume is made to 1000 ml with sterile double distilled water. 5. Alkaline Lysis solution I (Glucose, 50 mM; Tris-HCl, 25 mM, pH 8; EDTA, 10 mM, pH 8): Mix 5 ml of glucose (1M), 2.5 ml of Tris-HCl (1M; pH 8.0) and 2 ml of EDTA (0.5M, pH 8.0). The final volume is made to 100 ml with sterile double distilled water. Store at 4◦C after autoclaving. 6. Alkaline Lysis solution II (NaOH, 0.2N; SDS, 1%): Mix 2 ml of NaOH (10 N) with 10 ml of 10% SDS. The final volume is made to 100 ml with sterile double distilled water. Prepare solution fresh and use at RT. 7. Alkaline Lysis solution III (CH_3COOK, 5M; Glacial acetic acid): Mix 60 ml of CH_3COOK (5M) with 11.5 ml of glacial acetic acid and 28.5 ml of sterile double distilled water. Store the solution at 4◦C and transfer it to an ice bucket just before use.

5 Precautions:

1. Use autoclaved / sterilized glassware/plasticware. 2. Autoclave all the reagents except Tris – HCl solutions. 3. Store the reagents in the reagent bottles strictly at the prescribed temperatures. 4. Avoid the direct contact of skin with the ethidium bromide due to its mutagenic and carcinogenic nature. 5. Carefully handle Concentrated HCl. 6. Make sure that the pH of Tris and

EDTA solutions is 8. EDTA will dissolved completely only at pH 8. 7. The preparation of sodium hydroxide solution involves a highly exothermic reaction. So make this solution in a plastic beaker. 8. Lysozyme will not work efficiently if the pH of the solution is less than 8.0.