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KISHINCHAND CHELLARAM COLLEGE

DBT Star Scheme Activity

Department of Biotechnology

Presents

A MANUAL ON STANDARD OPERATING PROCEDURES

Compiled by: T.Y.B.Sc Biotechnology
2018-2019 Batch

ABOUT THE COLLEGE

Kishinchand Chellaram College is one of the premier institutions affiliated to the University of Mumbai and recognized by Government of Maharashtra. It was established in 1954 by the Hyderabad (Sind) National Collegiate Board. Motto of the College is 'SALVATION THROUGH KNOWLEDGE'. This motto was selected from ancient Sanskrit scriptures. 'Knowledge' in the highest sense means acquiring wisdom & understanding, which in turn leads man to "Moksha" or Salvation. K.C. prides itself in motivating students to achieve excellence not only in academic fields but also in co-curricular and extracurricular activities. With Dr. Hemlata Bagla as the Principal, the Signature Line of the College could well be – "Excellence - the only Compelling Option."

DEPARTMENT OF BIOTECHNOLOGY

Biotechnology is the third wave in biological science and represents such an interface of basic and applied sciences, where gradual and subtle transformation of science into technology can be witnessed. Biotechnology deals with the use of living organisms or their products to help improve our lives and the health of our planet. It is an old field that has been rejuvenated in recent years following the advent of genetic engineering techniques. At present, biotechnology is in an amazing growth phase whose end is nowhere in sight. Powerful new tools and technologies especially molecular genetics, diagnostic immunology, biochemistry, bioinformatics, promise exciting horizons for man's continued exploitation in science. Modern biotechnology provides breakthrough products and technologies to combat debilitating and rare diseases, alleviate our environmental footprint, have safer, cleaner and cost effective industrial manufacturing processes.

The Department of Biotechnology at Kishinchand Chellaram College offers a six semester undergraduate programme with the aim to prepare students for diverse careers ranging from research to teaching to industry. The department has constantly tried to keep students updated with the latest advances in technologies related to biological sciences. The department aims to ignite young minds and provide a strong foundation for their understanding in the rapidly growing field of biotechnology thus creating a pool of students that will be an asset to the Biotechnology Research community. as well as its associated industries.

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STANDARD OPERATING PROCEDURES
tell in writing about what should be done, when it should be done, where it should be done, and by whom it should be done

ACKNOWLEDGMENT

We, the T.Y.B.Sc students of Biotechnology take this proud moment to express our sincere and humblest gratitude to our institution Kishinchand Chellaram College under whose roofs have we been able to fulfill every task that we aim to take, including this.

We express our heartfelt gratitude to our dear Principal **Dr. Hemlata Bagla** for always inspiring us and encouraging us to work together and produce quality science.

We thank our Vice Principals; **Dr. Shailini Sinha, Dr. Smarajit Padhi & Dr. Mehak Gvalani** for their support and encouragement to every initiative that we choose to start.

We are indeed grateful to the **Department of Biotechnology (Government of India),** for their **STAR SCHEME** that has given us the opportunity to put this manual together and has also helped shape our writing and research skills.

We are deeply thankful to our Department Coordinator, **Dr. Sejal Rathod**, who has always guided us in our journeys and encouraged us to think big and better.

We could not have put this manual together without the support and constant review by our professors; **Ms. Sufiya Ansari** & **Ms. Hajra AS Gupta**. We would also like to thank our professors **Ms. Richa Shah, Ms. Prachi Bhatia,** and **Ms. Shruti Nair**.

We express our kind gratitude to our laboratory assistants; **Mr. Santosh Ganekar, Mr. Narendra Shirshat** & **Mr. Swapnil Raut** who helped our way out looking for instrument catalogs and technical details of each.

We spread and extend our love to each of our classmates who's worked on describing the working of every instrument in the laboratory diligently and helped build this manual that would educate the young and novice students walking in the department every year hereafter.

Thank you.

T.Y.B.Sc Biotechnology
Class of 2018-19

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AGAROSE GEL APPARATUS

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP01	**Agarose Gel Apparatus.**
Effective Date	01/09/2018	
Prepared by	Ms. Ashlesha Pande. Ms. Dhwani Sanklecha	
Approved by	Dr. Sejal Rathod Ms. Hajra AS Gupta	

PURPOSE

To provide a basic method for using agarose gel electrophoresis apparatus. A technique used for separation and analysis of macromolecules [DNA, RNA] and their fragments based on their size and charge.

SCOPE

This SOP covers all the procedures involving the use of the agarose gel electrophoresis apparatus. The goal of this SOP is to standardize how each task in the laboratory is performed by every student.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
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PRINCIPLE

A sample of DNA/RNA and proteins can be separated with the help of agarose gel electrophoresis apparatus. Gel electrophoresis refers to the technique in which these molecules are made to travel across a span of gel under the influence of electric current. Depending on the charge, the charged particle will migrate to their respective electrode. Migration of macromolecules also depends upon the molecular weight of the sample. Smaller the weight, faster the sample moves through the gel than the larger ones. Dyes are used in order to visualize the macromolecules. Common dyes used are ethidium bromide and SYBr Green. Further for illumination U.V. Transilluminator is used which visualize the stained nucleic acid.

MATERIALS

1. Agarose
2. 100 ml 1X TAE buffer (40 mM Tris, 1 mM EDTA, pH 8.0)
3. A DNA sample(s)

4. DNA loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol FF, 40% sucrose in water)
5. DNA ladder
6. Ethidium bromide staining solution (0.01g of ethidium bromide in 1 L of MilliQ H2O)
7. Destaining solution (1 X TAE or MilliQ H2O)
8. Gel tray and a gel caster
9. Gel comb
10. Horizontal electrophoresis unit
11. Horizontal electrophoresis unit lid
12. Power supply
13. Water bath 60*C
14. Glass flask (250 ml)
15. UV Transilluminator

ENVIRONMENTAL AND SAFETY CONTROLS

1. Proper fitted buttoned lab coat, closed shoes should be worn while working.
2. Hairs should be tied up
3. Heat resistant gloves should be used while handling the hot molten agar
4. Proper connection of the apparatus should be ensured before working
5. Gel chamber must have a lid or cover with safety interlocks to prevent accidental contact with electrodes.
6. Switch off all power supplies before opening the gel chamber lid
7. Wear appropriate skin and eye protection when working with U.V.Radiation.

PROCEDURE

The procedure is categorized into three parts:

A) Pouring a standard agarose gel:

1. 1g of agarose is measured.
2. The agarose is mixed with 100 mi 1*TAE buffer in the flask.
3. The solution is then kept in the microwave until the agarose is completely dissolved.
4. Cool down the agarose solution to about 50*C.
5. The EtBr dye is added to the solution.
6. The entire solution of agarose is poured into the gel tray with a good comb in place.
7. The gel is now allowed to settle either at 4*C for 15 mins or at R.T. for 30 mins.

B) Loading sample and running on agarose gel:

1. Loading buffer [0.25% bromophenol blue] is added to each sample.
2. Once the solidification has taken place, an agarose gel is shifted to the electrophoresis unit.
3. 1*TAE buffer is added until the unit has completely filled.
4. Molecular weight ladder is load in the first lane of the gel.
5. The sample is load into the additional wells.
6. Run the gel at 80-150V until the dye line is down the gel.
7. Carefully remove the gel from the unit after disconnecting the electrodes n switching off the power.
8. Visualize with the help of UV transilluminator.

C) Analysis of the gel:

Referring to the first lane of the ladder, the size of the band can be known.

TROUBLESHOOTING

Problems	Possible cause	Solutions
Faint or no DNA band	1. Insufficient quantity of concentration of DNA was loaded on the gel. 2. DNA was degraded.	• Increase the amount of DNA • Avoid nuclease contamination of DNA
Missing DNA bands	Small DNA bands were electrophoresed off the gel.	Electrophorese the gel for less time, at a lower voltage.

RELATED DOCUMENTS AND RECORDS

1. Instruction Manual.
2. Instrument Usage Log.
3. Calibration and Maintenance Log.
4. Warranty Card.
5. Amendment Log.

ABBREVIATIONS

1. TAE : Tris Acetate EDTA.
2. EtBr: Ethidium Bromide.
3. R.T.: Room Temperature.
4. SOP: Standard Operating Procedures.

AUTOCLAVE

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP02	**AUTOCLAVE**
Effective Date	01/09/2018	
Prepared by	Mr. Janav Panchal Mr. Holden Tuscano	
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PURPOSE

To provide a basic method for using an autoclave. An instrument used to sterilize and disinfect materials, media, and wastes (before disposal).

SCOPE

This SOP covers all the procedures involving the use of the autoclave.The goal of this SOP is to standardize how each task in the laboratory with respect to the autoclave is performed.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
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PRINCIPLE

Autoclaves serve as a method of sterilization of laboratory equipment and other media and materials. They work on the principle of moist heat under pressure [121 ℃ (250 ℉) at 100 kPa (15 psi) above atmospheric pressure for 15 minutes]**.** Autoclaves operate at these parameters to kill microorganisms and spores.

MATERIALS

- Bags and containers
- Heat insulated hand gloves
- Autoclave indicator (Verification strips)
- Biological indicators
- Lab coat
- Eye protection
- Closed footwear
- Materials/ media/ wastes to be autoclaved
- An Autoclave

ENVIRONMENTAL AND SAFETY CONTROLS

The *safety hazards* include:

- Skin burns from hot surfaces
- Steam burns
- Body injury in case of an explosion.

To ensure the safety of the personnel using the autoclave-

- A standard operating procedure should be posted near the autoclave.
- Proper training must be provided to all employees.
- Personal protective clothing must be worn at all times.
- Autoclaves must be inspected from time to time to check and validate perfect autoclaving for every cycle.

PROCEDURE

• Place the bag with all its contents – glassware, etc. in a shallow pan or tub.
• Place the all the contents into the autoclave, leave at least 5 inches of space above the water level.
• Run the autoclave at a temperature of 121°C for 20mins, at a pressure of 15 psi.
• Slowly open the autoclave after successfully releasing the pressure, protective gear must be compulsorily worn at this step.
• Remove all the materials and wastes. Discard the wastes in a black bag.

TROUBLESHOOTING

It is essential to know the limits of testing:

- Testing with a temperature indicator only tells you if the autoclave reached that desired temperature, but will not tell you how long that temperature was maintained.
- By making use of a device to check whether the autoclave is operating at the set temperature and pressure will not give accurate, reliable results on whether the materials have actually been sterilized properly.

  NOTE - Use a biological indicator strip to confirm whether the autoclave is maintaining its performance.

If the *autoclave does not operate as expected*, do not attempt to fix the problem.
Only qualified technicians are allowed to repair the autoclave.
One can note down the problems diagnosed in a log book and keep track of all the repairs made and the dates when servicing has been carried out.

CALIBRATION

Calibration ensures consistent results from a process.
Use a NIST-traceable device to calibrate an autoclave properly.
Calibration Protocol-

1. Record the calibration data – the gains and the zero.
2. Set the zero and gain to 0 and 1, respectively.
3. Use a NIST-traceable *dry block* and place the sensor appropriately.

4. Measure at the selected temperatures and record the standard and transducer data.
5. Carry out a regression analysis of the data you have collected
6. Carry out a correlation analysis as well. A straight line must be observed for a well-calibrated autoclave.
7. Enter the zero and gain values into the sterilizer controller to enter the calibration.
8. Verify the calibration using at least one point like your process temperature. If more than one process temperature, then verify at each one. Then you will have an exact statement of the accuracy of the sensors.
9. Repeat a couple of times.

RELATED DOCUMENTS AND RECORDS

- Instruction Manual
- Instrument Usage Log
- Calibration and Maintenance Log
- Warranty card
- Amendment Log

Records of repairs, service calls, and calibrations of autoclaves should be maintained by the facility in a log book.
Users must maintain records of any validation testing they perform on the autoclaves.
These records should be kept for the lifetime of the autoclave, or as long as feasible.

CELL FROST

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP03	**Cell frost (freezer)**
Effective Date	01/09/2018	
Prepared by	Ms. Monal Mehta Ms. Shalini Singh	
Approved by	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

Celfrost freezers are used in laboratories and hospitals for long-term storage and preservation of blood, specimen, bacterial cultures, enzymes, etc.

SCOPE

This SOP describes the optimal operation of the freezer (or deep freezer) achieved through proper installation and maintenance and relates to equipment in an infectious area of the laboratory

LOCATION

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PRINCIPLE

The freezer is intended to ensure a suitable environment for material that requires preservation at 17-24℃ or low.
The Freezer is insulated with high-density non-CFC polyurethane which helps in preventing the loss of temperature, thermal protection, and structural strength.
The refrigeration system comprises various components like compressor, condenser, evaporator, thermostat, etc., which are all enclosed in a casing which helps in the long-term preservation of samples and other materials.

SAFETY CONTROLS:

The freezer should be installed and operated in accordance with the specific manufacturers manual:

1. After transportation, leave the freezer in the upright position for some hours before connecting it to the electricity supply.
2. Do not install the freezer close to the heat sources
3. Locate the freezer in a dry, well-ventilated area.
4. Ensure that the freezer is placed on a level surface
5. In case of power failure, to prevent the loss of valuable cultures, chemicals, etc. the freezer should ideally be connected to an Uninterrupted Power supply system (UPS)
6. Never store flammable solutions in a freezer that is not approved and certified for this purpose

PROCEDURE

1. Install the cellfrost in a dry, level surface.
2. Connect the witch to the power supply
3. Wait for the freezer to reach the desired temperature
4. Place the samples and other biological materials contained in proper glassware
5. Do not overcrowd
6. Keep the bacteria, blood, specimens, enzymes indifferent sections/compartments

Defrosting:

1. Identify an adequate volume of available space in another freezer to store materials during the defrosting procedure. Ensure that the materials kept in the freezer are clearly marked
2. Switch the freezer off and disconnect it from the power supply. Leave the freezer door open.
3. Never use sharp tools to chip off the ice. Instead, use a container to collect it in.
4. Sponge and clean up any melted ice.

Cleaning:

1. Clean the interior of the freezer with a disinfectant or chemical sterilants like hydrogen peroxide
2. Clean the outside of the freezer with a soap solution (disinfectant) along with Dettol and dry it with a soft cloth
3. Reconnect the freezer to the main power supply and switch it on
4. Replace the original freezer contents once the required freezing temperature is reached. (DO NOT OVERCROWD)

Maintenance:

1. Daily: check the compressor for unusual heating or sound
2. Monthly: clean the filters and screens of the ventilator system with a brush or vacuum cleaner
3. Every 6 months: defrost and clean the freezer as described above.
4. Defrosting can be done more frequently if necessary, particularly in the event of leakage of biological materials onto the internal surface of the freezer or when the ice build up on the internal wall reaches a thickness of 4-6mm
5. Clean the condenser coils and fan with a brush or vacuum clean
6. Repairs should be performed only by a qualified service technician.

SAMPLES :

Typically a cellfrost freezer in labs is used to preserve:

1. Mycobacterial cultures and specimens
2. PCR Reagents, molecular biology reagents.
3. Antibodies, enzymes.

ABBREVATIONS

1. UPS = Uninterrupted Power supply
2. CFC = Chlorofluorocarbon
3. °C. = Degree Celsius

CENTRIFUGE

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP04	**CENTRIFUGE**
Effective Date	01/09/2018	
Prepared by	Ms. Sanjana Sawant Ms. Riya Debnath	
Approved by	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

To lay down a procedure to provide guidelines for the operation of the laboratory centrifuge.

SCOPE

This procedure is applicable for a centrifuge which is installed in a biotechnology laboratory.

LOCATION

3rd Floor, Biotechnology Laboratory,
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PRINCIPLE

A centrifuge is an instrument used for carrying out the separation of particles from a solution on the basis of size, shape, density, etc. It is a device that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis of spin (outward) that can be very strong. The centrifuge works using the sedimentation principle, where the centrifugal acceleration causes denser substances and particles to move outward in the radial direction. Simultaneously, objects with a low density are moved to the center. Upon centrifuging the sample tubes containing the sample, denser particles get settled at the bottom of the tube, whereas particles with the low-density move towards the top due to radial acceleration. There are various types of centrifuges available. The different parts of a centrifuge are a lid, centrifuge chamber, switch, motor, adjustment knob, and rotor.

Based on the rotor design the centrifuge used in the lab is a fixed angle centrifuge. The design of the fixed angle centrifuge is such that the sample is held at a specific angle

relative to the central axis. In a fixed angle centrifuge, the tubes are placed in cylindrical compartments in the rotor body. These compartments are set at a fixed angle from 14° and 40° to the central axis. The centrifugal field causes the particles to move in the radially outward direction, leading to the collision of the particles with the wall of the sample tube which leads to precipitation of the particles. Subsequently, a region of high concentration is formed with a density greater than that of the surrounding medium, due to which the precipitate aggregates at the bottom of the tube in the form of a compact pellet.

MATERIALS

1. Centrifuge tubes.
2. Source of electricity.
3. Rubber cushions.

ENVIRONMENTAL AND SAFETY CONTROLS

1. Ensure that the centrifuge is placed on a leveled and stable platform.
2. Ensure that the centrifuge tubes are balanced equally; otherwise, the centrifuge could vibrate and shake strongly.
3. Ensure rubber cushions for glass tubes.
4. Increase the speed gradually.
5. Bring the speed knob to OFF.
6. Do not open the lid during an ongoing centrifugation cycle.
7. Use only the rotors designed specifically for a particular centrifuge. Avoid interchanging the rotors. Check the rotor before using and if damaged, replace it.
8. Ensure that the tubes being used are certified for centrifuge usage and are compatible with the contents of the sample. The tubes used should be free of any damages such as cracks etc. The test tubes should be closed tightly before usage to avoid spilling of the sample.
9. Make sure the centrifuge chamber (bowl) and drive spindle, are free of scratches or other damage.
10. The centrifuge should never be operated without closing the lid.
11. If breakage of tubes is suspected and the sample is toxic or hazardous, then the user must not open the lid and should report the incident to the concerned authority to take the appropriate steps without causing harm to self, others, or the environment

PROCEDURE

1. The instrument must be clean and dust free before usage.
2. Ensure that the knob is in a normal position.
3. Place the centrifuge tubes in the cylindrical compartment in the rotor body.
4. Each tube can hold samples of up to 15 mL.
5. Ensure that the tubes are capped well.
6. The chamber is designed such as 8 tubes can be centrifuged at the same time.
7. Connect the centrifuge to the source of power and switch it on.
8. Use the speed adjustment knob to increase the RPM of the machine gradually.
9. A maximum RPM that can be reached is 3800.

10. This centrifuge does not have an automated timer and; hence, the switching off of the centrifuge must be done manually.
11. Keep the centrifuge on for the desired time and then switch it off.

TROUBLESHOOTING

Problem	Possible Causes	Check and/or Remedy
Imbalance	Incorrect loading of the rotor.	Balance your samples.
No spinning action	No rotor inserted.	Insert rotor.
	The rotor is not fixed correctly to the motor shaft.	Insert the rotor correctly. The pins have to align correctly with rotor slots.
Overheating	The temperature of the motor is too high.	Switch of the centrifuge for 15 minutes and then switch it on again.
Failure	Internal defect.	Call for servicing.
Accidental lid release	Lid opens accidentally.	Make sure the lid has been put securely. Change the lid if the problem persists.

RELATED DOCUMENTS AND RECORDS

1. Instruction manual
2. Instrument usage log
3. Calibration and Maintenance Log
4. Amendment log
5. Warranty card

ABBREVATIONS

1. mL: milliliter
2. RPM: Rotations per minute

COLD CENTRIFUGE

Department of Biotechnology		STANDARD OPERATING PROCEDURE COLD CENTRIFUGE OR REFRIGERATED CENTRIFUGE
SOP number	SOP05	
Effective Date	01/09/2018	
Prepared by	Ms. Riya Chheda Ms. Anuja Mestry	
Approved by	Dr. Sejal Rathod Ms. Hajra AS Gupta	

PURPOSE

Lay down a procedure to provide guidelines for the operation of the cold centrifuge.

SCOPE

This procedure is applicable to a cold centrifuge, which is installed in a biotechnology laboratory.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
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PRINCIPLE

The cold centrifuge is a device that uses the centrifugal force to separate the solid particles or liquids from a heterogeneous mixture or solution based on its size, shape, viscosity, and density .It can hold the sample tubes of different capacities which are made to rotate about a fixed axis. A centripetal force is generated due to the fast rotation which causes the heavier or denser substances to separate and settle at the bottom of the tubes by sedimentation. The rate of centrifugation is denoted in terms of revolutions per minute (RPM) or relative centrifugal force (RPF).The settling velocity of the particle during centrifugation depends on four main factors:

1. Size and shape of the particle.
2. Centrifugal acceleration.
3. The volume contained in the tube.
4. Density gradient of the particle and the liquid.
5. Viscosity.

It also provides the cooling mechanisms and helps to maintain a uniform temperature during experimentation and throughout the entire operation that uses the sample. The cold centrifuge is of immense importance in chemical, biological and biochemical experiments and research and is

extensively used for isolating and separating suspensions. Cold centrifuge works at a wide range of temperatures -20°C-40°C which makes them suitable for analysis of DNA, PCR, and antibodies. The basis of separation:

- Size: The lesser the size of the particle, the more the particle will be towards the base.
- Shape: Spherical particles settle down at the base faster than the polygonally shaped particles.
- Density-Denser, the particle, lower the setting.

MATERIALS

1. A suitable rotor
2. Sterile centrifuge tubes containing the experimental sample mixture
3. Laminar airflow
4. Suitable disinfectant (Cleaning the surface of the tube if needed), centrifuge tubes for balance if needed

ENVIRONMENTAL AND SAFETY CONTROLS

It is very important to keep the precautions in mind during handling of a cold centrifuge to prevent accidents. A cold centrifuge can pose two major types of hazards.

Physical hazards: Mechanical failure can often happen due to mishandling, and improper maintenance and is usually caused due to mechanical stress, metal fatigue or the corrosion of the rotor over time. It is important to replace the components of the centrifuge if they are damaged and regular checks must be carried out to ensure its smooth functioning.

Exposure hazards: Aerosol leakage of bio-hazardous, chemical or radioactive materials can occur during its operation. Care must be taken to seal any kind of leakage, and the lids of the tubes should be closed tightly to avoid spillage.

Emergency measures for Mechanical failures:

1. The centrifuge must be turned off immediately. To reduce the aerosol leakage of harmful chemicals the lid of the centrifuge must be kept closed for a minimum of 10 minutes. Putting a 'SPILL' sign is essential to let the other laboratory technicians be aware of it.
2. Use of appropriate personal protective equipment is absolutely necessary. This includes gloves, safety glasses or goggles, face shield, lab coat, and closed toe shoes.
3. The bench-top unit must be shifted to the biosafety cabinet or chemical fume hood in order to clean up the spill. In the case of larger centrifuge machines, only the rotors can be moved.
4. The contaminated protective equipment after its use which includes the gloves and cleaning equipment must be placed in the hazardous waste stream.
5. It is essential to wash hands and any exposed skin surfaces properly with soap and water as they may carry harmful chemicals. One must inform the occurrence of the incident to the laboratory supervisor.

Precautions for handling bio-hazardous materials:

1. The tubes and bottles must be checked for any cracks or stress marks before they are used. Any centrifuge tubes having cracks must be discarded.
2. The surface of the centrifuge tubes must be wiped with a suitable disinfectant when working with bio-hazardous materials before removing them from biosafety cabinet and also before placing in the centrifuge rotor.

3. Seal the rotors before the centrifuge is switched on. It is also important to balance the weight of the tubes in the rotor before they start spinning. It must be equal on opposite sides of the rotor.

4. Any spills or leakage if observed in the centrifuge rotors after or before use must be cleaned with a mild detergent followed by rinsing thoroughly with distilled water .Air dry it until it is completely dry on the insides.
5. The rotor and centrifuge must be cleaned after each use.

PROCEDURE

1. The instruction manual must be read before starting the use of the centrifuge, and it must be used according to the study protocol
2. Protective wear is a must while working in a laboratory and while handling laboratory equipment. It is essential to wash one's hands before handling the blood samples as mentioned in the STH hand hygiene policy.
3. When there is a risk of contamination, use of disposable gloves is highly recommended.
4. One must ensure that the centrifuge is switched on at the wall plug socket before use and switched off after its use.
5. The lid of the centrifuge should be kept open when it is not being used. The lid must be opened fully so the inside of the centrifuge can be seen.
6. While placing the samples in the centrifuge, one must first unscrew the lid of the rotator bucket and then place the centrifuge tubes containing samples opposite to each other in swinging bucket adaptors present on the rotator bucket.
7. Before running the centrifuge, it is necessary that the rotator buckets and the caps of the centrifuge tubes are closed firmly .The lid of the centrifuge must also be tightly placed.
8. The centrifuge should be balanced with equal volume and weight of the samples on each side. A useful technique in balancing is placing the equal weight of the centrifuge tubes containing samples with an equal volume on the opposite ends of the rotator bucket. An additional centrifuge tube containing an equal volume of distilled water can also be used for balancing the weight.
9. The time and the speed of the rotation can be set using the “set” key on the time/rotor field which is located in the front panel and the scroll up or down button consisting of the “+” and “–“ keys can be used until the desired time is reached. Press “set” to program the time which will be displayed in red on the front panel.
10. While using the centrifuge, the speed is always set gradually starting from and reverting to zero .This will help to resolve any defect in the centrifuge .During an emergency ,the stop/pause button is pressed after which the device slows down to a stop, and the door lock is released .
11. The temperature must be set 10 minutes before use after placing the refrigerated centrifuge samples, and the lid is closed.
12. The centrifuge should never be left uncovered in working conditions or in the refrigerated mode when left unattended .This is very dangerous as it will cause the refrigeration mechanism to break.
13. The centrifuged samples must only be taken out after the centrifuge stops completely and the speed returns to zero.

TROUBLESHOOTING

1. The work surface must be level and firm. Do not centrifuge on an uneven or slanted work surface.
2. Balance the tubes in the rotor. To use10ml of liquid sample place another 10 ml of water in the opposite hole on the rotor. If the liquid has a higher or lower density than water, you must balance the tubes by mass and not volume.
3. Do not open the lid when the rotor is moving. Even though many centrifuges have a safety shutoff, if the lid is opened, the only thing this does is stop powering the rotor. The rotor will still spin due to its own inertia until friction slows it down and eventually stops it.
4. If you see wobbling or shaking, turn it off and pull the plug. A little vibration is normal, but excessive amounts can mean danger. Double check if the centrifuge tubes are correctly balanced .If the wobbling still continues, contact the manufacturer or dealer. Do not try to run the centrifuge which is wobbling while the rotor is moving.
5. Wear a face shield or safety goggles if you are working near the centrifuge.
6. Do not bump or move the centrifuge when the rotor is spinning .Make sure there is no cord that is dangling from the edge of the table where the centrifuge is placed, or it might catch someone's feet and pull down the centrifuge damaging it.
7. It is necessary to maintain the centrifuge and carry out regular checks to ensure that it works efficiently.

Daily maintenance	**Monthly maintenance**	**Annual maintenance**
Rinse thoroughly and wipe insides of the centrifuge with disinfectant	Clean the rotor accessories, rc chamber and centrifuge hous with a neutral cleansing agent	The centrifuge must be servi annually by a qualified technic to ensure safe operation.
A qualified service technician m be contacted if there is unus noise or if parts of the centrifuge damaged	Clean plastic and non- metal pa with a fresh solution of 5%sodi hypochlorite(bleach)mixed 1 with water	Condenser coils, fans, scree filters, brushes, bearings, tim speed, temperature, and electri integrity must be checked, and inspection certificate must issued

RELATED DOCUMENTS

1. Safe working and prevention of infection
2. REMI operating instructions
3. REMI hand hygiene policy
4. Project delegation log
5. REMI infection control guidelines

COLORIMETER

Department of Biotechnology		STANDARD OPERATING PROCEDURE COLORIMETER
SOP number	SOP06	
Effective Date	01/09/2018	
Prepared by	Ms. Ayesha Zarodarwala	
Approved by	Dr.Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

The following S.O.P describes the procedure for operation of EQUIPTRONICS DIGITAL COLORIMETER EQ-650.

SCOPE

This instrument is used to measure the absorbance of light passing through a solution.

LOCATION

3rd Floor, Biotechnology Laboratory,
KishinchandChellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

The colorimeter works on the principle of Beer-Lambert's law which states that 'When a beam of monochromatic light passes through a solution, the amount of light absorbed by the substance is directly proportional to the concentration of the substance and the path length of light passing through the solution.

$$A = \varepsilon cl$$

Where;

A- Absorbance of light
Ɛ- Molar absorptivity constant/ Molar extinction constant
c- Concentration of sample
l- Path length of light.

When a beam of light of known wavelength passes through a solution via a series of lenses in a colorimeter, some of the light is absorbed by the substance present in the solution. The amount of light absorbed depends upon the concentration of solute in the solution.

The concentration of an unknown sample can be determined using a colorimeter. Various solutions of known concentration are prepared, and absorbance is measured using a colorimeter. Then a graph of absorbance vs. concentration is plotted, and the concentration of unknown sample solution is obtained from the graph.

MATERIALS

1) The cuvette of path length 1cm
2) Filter paper

HANDLING AND STORAGE

1. Remove colorimeter safely from the cupboard.
2. Check that the area surrounding colorimeter is clean.
3. After use place it back in the container and keep it inside the cupboard.

PROCEDURE

1. Switch ON the instrument.
2. Switch on the instrument at least 10 minutes before use and to allow it to stabilize.
3. Move the filter wheel and select the desired wavelength (Range 400nm-700nm)
4. Place the mode selector at %T and adjust the transmittance to 100 using ADJUST 100% knob.
5. Press the mode selector again and switch to absorbance mode.
6. Adjust absorbance to 0 using the knob.
7. Fill the cuvette with distilled water or a solution used as a blank.
8. Clean the outer surface of the cuvette using filter paper.
9. Insert the cuvette filled with a blank solution in the colorimeter.
10. Adjust the absorbance to 0 using knob.
11. Remove the cuvette and place it in cuvette holder.
12. Fill the cuvette with the sample solution.
13. Clean the outer surface using filter paper.
14. Insert the cuvette filled with a test sample in colorimeter and record the results.
15. After obtaining results, discard the cuvettes containing blank and sample solutions and rinse with water.
16. Switch off the power button after use.

CALIBRATION

The method adopted to calibrate a colorimeter is by the use of a standard solution of known molar extinction coefficient (Ɛ)
Molar extinction coefficient value of the standard solution is determined using a colorimeter.
Difference between the expected and the obtained value is calculated.
If the error value is more than 0.01 O. D, then the instrument is sent for recalibration to the supplier.

- PROTOCOL:
- PART 1: Determination of λ_{max} and molar extinction coefficient (Ɛ).
    - ✓ Take the 0.02M KMNO4 solution

- ✓ Use D/W as blank
- ✓ Measure the absorbance at a different wavelength.
- ✓ Plot a graph of absorbance vs. wavelength and determine $\lambda_{max.}$
- ✓ Calculate the value of the molar extinction coefficient and compare with the standard value of the molar extinction coefficient.

- PART 2: Beer Lambert's law: Determination of molar extinction coefficient (Ɛ).
  - ✓ Prepare different dilutions of the 0.02M KMNO4 solution.
  - ✓ Take O.D values of diluted samples.
  - ✓ Plot a graph of absorbance vs. concentration and determine the molar extinction coefficient.

TROUBLESHOOTING

1. In order to prevent the error caused due to electronic variations, switch on the instrument and let it get warmed up for 10 minutes prior to its use.
2. Check that the cuvette is filled up to three-fourths with the solution, including the blank so that the beam of light of appropriate wavelength travels through the solution.
3. Check that the cuvette is placed in colorimeter in such a way that light beam passes through the clear sides of the cuvette.
4. Use the same cuvette to measure the absorbance of various solutions for a given experiment. Use cuvettes with a path length of 1cm.
5. If the colorimeter is calibrated at a wavelength, then it has to be recalibrated again if the wavelength is changed.

RESPONSIBILITY

a. The staff should train students about the proper usage and handling of the colorimeter.
b. The students should follow SOP and inform the instructor about problems occurring during usage of this instrument.

DISTILLATION UNIT

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP07	**DISTILLATION UNIT**
Effective Date	01/09/2018	
Prepared by	Ms. Neeti Kothari	
Approved by	Dr.Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

To lay down the procedure for operating the Distillation Unit.

SCOPE

This SOP is applicable to the distillation unit installed in the biotechnology lab.

LOCATION

3rd Floor, Biotechnology laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal,
K.M Kundnani Chowk,
D.W Road,
Churchgate.
Mumbai - 400020

PRINCIPLE

Distillation unit is an assembly which purifies water by simple means of boiling and condensation. The water is boiled, and the vapors are arising are then condensed to obtain a fraction of distilled water which is free from impurities. Since the boiling point of impurities in the water is different from that of water, only pure water is collected in the container.

MATERIALS

1) Water to be purified
2) Heating device
3) Condensing system
4) 3 pipes

Reagents: Concentrated HCl

PROCEDURE

1. The Distillation unit has 3 pipes :
   a) One which is connected to the water inlet.
   b) Second from which distilled water would be collected.
   c) From the third pipe, wastewater would be discarded.
2. Connect the water inlet pipe to the tap
3. Switch on the main supply
4. Keep an empty, clean water container below the water outlet pipe to collect the distilled water
5. Switch on the unit
6. Collect about 500 ml of Distilled water initially and discard it. Then collect the required amount of Distilled water in a container.
7. Analyze the distilled water every week.
8. The distilled water collected can be used in the lab, organic chemistry lab,clinic, fermentation, and medical industry.

TROUBLESHOOTING

1. The connections of the pipe to the main supply should be checked thoroughly.
2. If the water supply is extremely slow, this could create a hindrance in the distillation process.
3. Due to the cleaning of the distillation unit with conc reagent, there could be an accumulation of limescale which has to be removed for optimal performance.
4. Cleaning is performed with concentrated HCl, so care has to be taken that at the end of the operation the acid has been neutralized. So necessary precautions have to be taken.

CALIBRATION

1. There should be no leaking of water from the main connection.
2. The drain water should flow freely.

PEOPLE RESPONSIBLE

1. Unit operator
2. Supervisor

RELATED DOCUMENTS

1. Manufacturers' manual specific to the distillation unit.
2. Maintenance manual for lab equipment
3. Main log book
4. Amendment book

HOT AIR OVEN

Department of Biotechnology		STANDARD OPERATING PROCEDURE HOT AIR OVEN
SOP number	SOP08	
Effective Date	01/09/2018	
Prepared by	Ms.Piroza master Ms.Juveriya Jawahire	
Approved by	Dr. Sejal Rathod Ms. Hajra AS Gupta	

PURPOSE

To provide a basic method for the setting up a hot air oven, an electrical device that is used for the sterilization procedure.

SCOPE

This SOP covers all the procedures involving hot air oven. The goal of this SOP is to standardize how each task in the laboratory is performed by every student

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

Hot air oven is widely used to sterilize equipment and materials used in the medical field or for the microbiological procedures.A hot air oven worked on the principle of dry heat sterilization and is used for materials which will not melt, catch fire or change forms when exposed to high temperatures. They use extremely high temperatures over a specified period to destroy microorganisms and bacterial spores. The oven uses conduction to sterilize items by heating the outside surfaces of the item, which then absorbs heat and moves it towards the center of the item.

The commonly used temperatures and time that hot air ovens need to sterilize materials are 170^0Cfor 30 minutes, 160^0C for 60 and 150^0C for 150 minutes

MATERIALS

Items that are sterilized using a hot air oven include:

1. Glassware (Petri plates, flasks, pipettes, and test tubes).
2. Powders (starch, zinc oxide and sulfadiazine).
3. Oils.
4. Metal equipments (scalpels, scissors and blades).

PROCEDURE

a. Ensure the cleanliness of the instrument.
b. Open the ventilation knob provided on top of the oven.
c. Switch on the power supply.
d. Electronic temperature controller displays the chamber temperature.
e. Set the required temperature by pushing the 'PUSH' switch and the first potentiometer knob clockwise or anticlockwise until the temperature comes to set one.
f. Set the temperature with the help of the second potentiometer knob.
g. Release the 'PUSH' switch.
h. Indicator bulb glows which indicates that the power to the heater is on.
i. Switch on the fan switch for air circulation.
j. Use a rotary switch for precise control of temperature.
k. Four positions of Rotary switch are available as follows:-
    i. Off position
    ii. 5 degree Celsius above ambient to 90°C,
    iii. 90 degree Celsius to 150°C
    iv. 150 degree Celsius to 250°

The time and temperature protocols are as listed:

Temperature	**Time**
130 Degree Celsius	2 hours
160 Degree Celsius	1.5 hours
190 Degree Celsius	15 minutes

(Above temperatures and time can be set as per required)

PRECAUTION AND SAFETY CONTROL

a. Ensure that the equipment is on a firm base.
b. Do not keep any glassware containing media or with organic solvents into the hot air oven.
c. Do not increase the temperature more than required.
d. The equipment should be clean and suitable to use.
e. Ensure that the fan is working properly.
f. The glassware should be plugged with cotton wools.
g. Overloading of the hot air oven should be avoided. There should be enough space between the materials to ensure free air circulation

TROUBLESHOOTING

Problem	Possible Causes	Check and/or Remedy
Heating does not take place.	Faulty igniter.	Connecting a new igniter, disconnecting the range from power and wiring and then connecting them back.
Parts loosen and wear out.	The instrument has aged due to long time storage	The instrument needs servicing.
Heating is not reaching the correct temperature.	The sensor is not working.	The sensor should not touch the inside wall, repositioning of the sensor or replacement of the sensor.

CALIBRATIONS

a. All the essential components of the hot air oven should be calibrated.
b. Calibration frequency is once a year by some external party or after major maintenance or repairs.

INCUBATOR

Department of Biotechnology		STANDARD OPERATING PROCEDURE INCUBATOR
SOP number	SOP09	
Effective Date	01/09/2018	
Prepared by	Ms. Rekha Chataule	
Approved by	Dr. Sejal Rathod Ms. Hajra AS Gupta	

PURPOSE

The purpose of this procedure is to provide a method to standardize the operation and monitor the temperature of the incubator; an instrument used to maintain the biological cultures and to provide means of documentation.

SCOPE

The SOP covers all the procedure for operation of the incubator. The goal of this SOP is to standardize the procedure to how to set and use the incubator at a temperature for microbial cultures.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

The incubator is a laboratory instrument used to maintain bacterial cultures. Incubator maintains the optimal temperature, humidity and mimics the conditions required for the growth and the maintenance of bacterial cultures. Incubator plays a very important role in the fields of biotechnology, microbiology, molecular biology, etc. as it provides a contaminant-free environment required by the bacterial cultures. The optimal temperature used to maintain and grow bacterial cultures is 37 degrees Celsius. It also has applications in the poultry industry wherein incubating eggs hastens their hatching process.

COMPONENTS

1. The incubator has double walled cabinets made up of mild steel which is logged with insulation material.
2. The inside of the instrument is made up of clear glass fitted in a metal frame, and the junctions are sealed with a gasket.
3. The outdoor of the instrument is made up of mild steel and double walled.
4. The instrument has adjustable shelves made up of crimped wire mesh.
5. The heater is fitted at the base of the instrument along with a thermostat.

PROCEDURE FOR USE

1. Place the bacterial cultures (slants, plates, flasks, etc.) on the shelves.
2. Firmly close the doors.
3. Adjust the required temperature, i.e., 37 degrees Celsius.
4. Keep the bacterial cultures for the given incubation period required by the bacterial cultures.
5. After incubation period remove the bacterial cultures.

TROUBLESHOOTING

Problem	Possible Causes	Check and/or Remedy
The display for temperature not glowing properly.	Main power supply and electrical connections not connected properly.	Make sure that the main power supply and electrical connections of the incubator are connected with the power point.
The temperature is not working properly.	Air circulating fan not functioning properly.	Clean the air circulating fan properly and check for its connection.
Any discrepancy observed.	Incubator not checked in regular intervals of time.	Report to quality control any discrepancy observed during any operation or monitoring temperature and made the Maintenance Department notify the defect.

CARE AND MAINTENANCE

1. Check the incubator often to make sure it is contaminants free.
2. Clean the incubator often once in a while.

RELATED DOCUMENTS AND RECORDS

1. Instruction Manual
2. Instrument Usage Log
3. Calibration and Maintenance Log
4. Warranty Card
5. Amendment Log

MICROPIPETTE

Department of Biotechnology		STANDARD OPERATING PROCEDURE MICROPIPETTE
SOP number	SOP10	
Effective Date	01/09/2018	
Prepared by	Ms. Anjaly Tankaria Ms. Sakshi Shinde	
Approved by	Dr.Sejal Rathod Ms.Sufiya Ansari	

Purpose

Lay down a procedure to provide guidelines for the operation of the micropipette.

Scope

Applicable to Microbiology and Biotechnology department.

Location

3rd floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar principal K.M. kundani chowk
D.W. road,
Church gate,
Mumbai-400 020

Principle

The volume of any sample can be calculated by measuring dispensed water. Gravimetric analysis is the technique used for the quantification of the pipette. To regulate the volume of water or any sample, the exactness is forced by ambient temperature, atmospheric pressure, and relative humidity. The combination of these factors will grant 'Z' factor which is useful in the deliberation of the volume of water. The presumed volume is contrasted with the determined volume of water to evaluate the exactness of pipette.

Materials: sample liquid, functional micropipette [of desired range], micropipette tips, cotton, disinfectant [Dettol], a container with detergent to discard micropipette tips, stand.

Environmental and safety control

- Always wear gloves to avoid contamination.
- The pipette should be placed down if it contains a solution.
- To avoid contamination, disallow any other substance to come in contact with the liquid.

Procedure

- Volume settings: By altering the ambiance of the ring, the volume can be changed frequently.

- Pipette tips: unless and until the pipette ring does not adhere to pipette, it cannot be useful.
- Aspirating liquid:
  1) the sample to be measured is arrested from a liquid
  2) Append the tips to the pipette firmly.

3) Submerse the pipette into the sample.
4) Allow the back button to control back slowly.
5) Drack the micropipette tips away

Dispensing liquid:

1) The tips of the pipette should be held at a certain angle against the solid support.
2) The control button should be held, and the sample should be allowed to dispense until the last drop.
3 The control button should slide up slowly

Troubleshooting

Fault

- Inaccurate dispensing
- Contamination
- Drop left inside the tip
- Push button jammed
- The volume button is not properly clicked or over rotate

Possible damage

- Tip incorrectly attached
- Overuse of the pipette
- Unsuitable tip/instrument damaged
- Penetration of solvents
- Tip contaminated from outside/mechanism damaged

Calibration

- Calibration should be done every 3 months.
- Different volumes of the sample and should be performed linearly.
- The estimation should be done by using set volumes.
- Calibration of the micropipette should be done in a vibration-free room and at room temperature.

Responsibility

- Microbiologist/ Chemist/Biotechnologist.

MICROSCOPE

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP11	**MICROSCOPE**
Effective Date	01/09/2018	
Prepared by	Ms. ArantaDongre	
Approved by	Dr. SejalRathod Ms. Sufiya Ansari	

PURPOSE

To provide a basic method for using a microscope, also to describe the general protocol for the use, calibration, and maintenance of the microscope in a biotechnology lab.

SCOPE

This SOP covers all the procedures involving the use of a microscope. The goal of this SOP is to standardize how each task in the laboratory is performed by every student.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. KundananiChowk,
D. W. Road,
Churchgate,
Mumbai- 400020

PRINCIPLE

A microscope is an optical instrument used to view objects in the micron size range. The sample is illuminated with visible or ultraviolet light using an optical source. The microscope is categorized as inverted or upright depending upon its structure and magnification range.To obtain the desired level of different magnification microscopes are used. For low magnification magnifying glass is used for inspection, to observe from 10x to 50x binocular microscopes are used, while upright and inverted microscopes are used to observe from 50x to 1500x.

MATERIALS

1. A microscope fitted with 10x oculars (eyepiece) 10x, 40x and 100x objectives and a mechanical stage.
2. Cedarwood oil or liquid paraffin
3. Coverslips
4. Glass slides
5. Microscope cleaning solution with a soft cloth.

ENVIRONMENTAL AND SAFETY CONTROL

1. Fungal growth is easily established on a lens or a prism, in warm, humid climates. Therefore microscopes should be stored in dry conditions when not in use to prevent fungal growth. Example- storing in a dark cupboard.
2. The power source and connections to the microscope should be secured and stabilized and not exposed to the risk for electrocution.
3. The microscope and the electrical connections should not be exposed to water.
4. Care should be taken to prevent eye damage, from exposure to high light- intensity of halogen lamps.
5. The microscope should be used ergonomically to prevent neck and back strain.

PROCEDURE

1. Turn the revolving turret, so that the lowest power objective lens, that is 10x is in its position.
2. Place the microscope slide on the stage and fasted it with stage clips.
3. Look at the objective lens and the stage; turn the focus knob so that the stage moves upward. Move it up as far as it goes without letting the objective touch the coverslip.
4. Looking through the eyepiece, move the focus knob till the images come into focus.
5. The greater amount of light is obtained by adjusting the condenser.
6. Move the microscope slide around until the sample is in the center field of view. (what we see)
7. The focus knob is used to focus the sample, and readjust the condenser and light intensity for clearest image.
8. When the clear image of the sample is obtained at (40x & 100x) objective lens, then it can be changed to the next objective lens (Readjustment of the samples focus, or the light intensity or condenser should be done.
9. To observe microorganisms, 100X or oil immersion lens is used.
10. Place a drop of cedarwood oil on the specimen and begin to focus.
11. Adjust the coarse adjustment knob to locate the smear.
12. With the fine adjustment knob, sharpen the image to identify the morphology & character of the organism correctly.
13. After noting the observations, lower the stage.
14. Remove the slide and discard it.
15. Clean the oil immersion lens with xylene dapped filter paper.
16. When finished, lower the stage, click the low power lens into position and remove the slide.
17. Switch off the microscope and pull the plug out.
18. Return the microscope in the locker table.

TROUBLESHOOTING

COMMON FAULTS	POSSIBLE CAUSES
No light	• Power cord has not been connected; power is switched off.' • The wrong bulb is installed. • Light intensity control is turned down too low. • The objective is not properly in position. • The bulb has burnt out.
The image is too dark	• Increase the intensity of light • The substage iris diaphragm is not open enough • The condenser is too low.
The image is too light	• Decrease light intensity. • The sub-stage iris diaphragm is open.
A spot in the field of the view, which doesn't move when the slide is moved.	• The lens is dirty. Clean the eyepiece and objective.
Poor image quality, poor resolution, image not sharp.	• Clean objective, eyepiece, and a condenser. • Check if immersion oil is contaminated or cloudy or air bubble are present. • Slides are always the wrong way up.
Uneven illumination	• Adjust the condenser • Make sure objective has clicked into place.
Flickering light	• The bulb needs loose replacement connection at the outlet. • Bulb not properly inserted. • Check the voltage supply.
Half the viewing field is illuminated	• Make sure the objective is clicked into place
Unable to focus the slide	• Coverslip is too thick. • The slide is the wrong way up. • The stage is slowly dropping, adjust the tension of the coarse focus knob. • Clean the slide, objective and eyepiece.

CALIBRATION

1. Remove the eyepiece and insert the ocular micrometer.
2. The graduations in the ocular micrometer can be seen under sharp illumination.
3. The lines and distances will remain unchanged under different objectives.
4. The stage micrometer is mounted on the microscopic field under sharp focus.
5. This is done first with low power objectives and then under high power objective.
6. The scale of ocular micrometer and stage micrometer is adjusted in such a way that the lines of one should superimpose on the lines of other at one end of the microscopic field.

7. The number of divisions is counted lying in between two coinciding divisions of both ocular and stage micrometer.
8. Since we know the value of each division on stage micrometer the value of division on the ocular can be calculated.

RELATED DOCUMENTS AND RECORDS

1. Instruction Manual
2. Instrument Usage log
3. Calibration and Maintenance Log
4. Warranty card.

ABBREVATIONS

1. SOP – Standard operating procedure
2. NO – Number
3. QC – Quality Control
4. QA – Quality Assurance

MICROWAVE

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP12	MICROWAVE
Effective Date	01/09/2018	
Prepared by	Ms. Sarrah Rampurawala Mr. Anuj Singh	
Approved by	Dr. Sejal Rathod Ms. Hajra AS Gupta	

PURPOSE

To provide a basic understanding of the procedure and the risks involved in the operation of a Microwave Oven. This method may be modified according to the parameters required in any particular household depending on the model and brand of Microwave Oven.

SCOPE

This SOP covers all the procedures involving the Microwave Oven. The goal of this SOP is to understand how the main functions are performed by every individual.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

Food Molecules such as Water, Fat, and Sugar can be excited by the high occurrence of microwaves that are generated. For a molecule to be excited the electrons “orbiting” the nucleus have to jump up energy levels. When this occurs, the atom starts to vibrate faster than normal. When this happens in a glass of water, for example, all the atoms that make up water start to move and run into each other and create friction. When friction is created, energy is given off in the form of heat. Microwave Ovens use this concept of Heat generation to reheat food.

MATERIALS

1. Microwave
2. Gloves
3. Apron
4. Microwave resistant dishes
5. Oven Mitts
6. The worker needs to be appropriately dressed in closed slippers which are water resistant and provides adequate support.

PROCEDURE

Preparation:

- It is important to clean the microwave properly with a semi-wet cloth to ensure that it is free from any kind of particles.
- The substance to be heated needs to be covered before heating to prevent spraying.
- Suitable microwave glass dishes should be only used as they are able to withstand the temperature of the microwave.

Microwave use

- Appropriate temperature and time need to be set up before the substance is placed in
- While the substance is being heated up the microwave door needs to be tightly shut to avoid any accidents.
- It is important to wait for 3-6 seconds before opening the oven door after the substance is heated.

After use

- To remove the substance out of the oven mitts, need to be used. The container may not be as hot as the material inside it therefor one needs to be extremely vigilant while taking out liquids from the oven.
- There will be rapid steam evasion when lids and foils are opened.
- The tray placed on the lower end of the oven needs to be cleaned adequately after every use.
- Sanitization of the outer and inner region of the oven is crucial.

TROUBLESHOOTING

Problems	**Checks and/or Remedies**
Burns	Substances that are combustible should not be heated in the microwave. Aluminum foil and metallic dishes should be evaded. If a situation arises such that a fire breaks out, switch off the oven instantly and keep the microwave door shut till the fire is out.
Food Contamination	All the appliances used should be well sterilized. Substances should be heated up to proper temperatures given the specific time set. It is important to change gloves regularly.
Manual Handling	The oven should not be placed above shoulder height; it cannot be placed even below bench height.

PCR MACHINE (THERMOCYCLER/ THERMAL CYCLER)

Department of Biotechnology		STANDARD OPERATING PROCEDURE PCR MACHINE (THERMOCYCLER/ THERMAL CYCLER)
SOP number	SOP13	
Effective Date	01/09/2018	
Prepared by	Ms. Andrea S Pinto	
Approved by	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

To provide a basic method for setting up a PCR reaction, a technique for specific amplification of a short, defined segment of DNA. This method may be modified according to the individual parameters required in any particular experiment.

SCOPE

This SOP covers all the procedures involving Polymerase Chain Reaction (PCR). The goal of this SOP is to standardize how each task in the laboratory is performed by every student.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

A DNA or RNA sample can be amplified by polymerase chain reaction using instruments such as Thermocyclers or Thermal Cyclers. The instrument works by raising and lowering the temperature of the samples in the holding block. A PCR reaction involves three major steps: denaturation, annealing, and extension of the sample. All the major steps are pre-programmed into the Thermocycler. After the extension step, the

Thermocycler can hold the PCR tubes at a lower temperature for an indefinite time; however, the sample is withdrawn and preserved at a lower temperature. The amplified genetic material can then be used for gene sequencing, genotyping, cloning, expression analysis, etc.

MATERIALS

1. DNA Template
2. PCR supermix/ Readymix RedTaq PCR Reactive Mix
3. Molecular Biology grade sterile D/W
4. Primers - working standard: 100µM concentration each of forwarding and Reverse Primer
5. Applied Biosystems Thermal Cycler 2720
6. Sterile PCR tubes in a beaker
7. Sterile 1.5ml microfuge tubes in a beaker
8. Sterile 1µl, 100µl, and 1000µl tips and respective micropipettes
9. Disposable 96 slots plastic tray
10. Centrifuge to spin down the samples
11. Freezer at -20ºC

ENVIRONMENTAL AND SAFETY CONTROLS

1. All the glassware and the reagents used in the PCR reaction have to be sterilized and should be carried out under sterile conditions.
2. All the reaction mixture tubes should be stored on an ice bath.
3. Use a disposable plastic tray.
4. Do not touch the heating block on the PCR machine where you place the tubes. It is very hot and may lead to skin burn.
5. To standardize the annealing temperature, carry out step up temperature method to avoid primer-dimer bonds and amplification of the undesired DNA fragment.
6. Use sterile gloves while performing the PCR reaction to avoid contamination.
7. Do not use Ethidium Bromide for Agarose Gel Electrophoresis (AGE) without gloves as it is a carcinogen. Therefore careful precautions must be taken for its disposal.

PROCEDURE

1. Swab the work table first with Dettol and then with ethanol. Thaw the PCR reaction mixture, sterile D/W, primers and template and store it on an ice bath.
2. Label one sterile microfuge as MM-Mastermix. Accordingly, label the rest tubes in serial order. Calculate the amount of each reagent that is required for each reaction tube. Accordingly, calculate the amount that has to go in the master mix tube. It depends on the number of PCR tubes that has to be prepared.
3. Add each component (RedTaq PCR ready mix, forward and reverse primer, sterile D/W) to the mastermix aseptically. Dispense the required amount in each of the PCR tubes.

4. Add the template directly in their respective PCR tubes.
5. Spin down the tubes and store the PCR tubes on an ice bath.
6. Press the power on/off switch at the rear of the instrument. A whirring fan sounds and the startup screen appears.
7. To add yourself as a user, from the main menu, press F5 (user) and enter your name along with a password. Press F1 (accept) to save it.
8. To create a method, press F2 (create) from the main menu. Program the Thermal Cycler as follows:

Steps	Temperature	Time
Denaturation	94ºC	1 minute
Annealing	52ºC	2 minutes
Extension	72ºC	3 minutes
1st hold	72ºC	10 minutes
2nd hold	4ºC	Infinity

(Above temperatures and time can be set as per required)

9. Enter the number of cycles required.
10. Press F2 (store) to save the method and press F3 (method) to enter the method name.
11. Press F1 (accept) to save the method along with the name. The system saves the method and returns to the main menu.
12. Place the microfuge (PCR) tubes in the disposable plastic tray, preferably in the center portion, leaving space in between each tube. Place this rack on the heating block of the Thermal Cycler and close the lid.
13. From the main menu, press F1 (run) and select the program name that you created earlier.
14. Press F1 (start) and enter the reaction volume.
15. Wait for the heated cover to reach 103ºC. After this, the 1st cycle of the PCR reaction begins.
16. To find out when the run will end, press F4 (info).
17. To pause a run press F1 (pause) and to stop a run press the stop button.
18. When the screen shows 4ºC, it indicates the program has ended. Press the stop button twice to abort the function.
19. Place the tubes on an ice pack if it has to be tested immediately by AGE or store it at -20ºC till later use.

TROUBLESHOOTING

1. If power is interrupted, the instrument does the following:

- Restarts or continues the PCR experiment.
  The Thermocycler determines the temperature that was being achieved or the holding temperature. When the power supply is resumed, it returns to that temperature and estimates the remaining time in the hold as soon as the temperature is within the range of the specified start time.
- Incubates the samples until such time as the experiment can be continued.

- Enters a record for any power outage in the history file.

Problem	Possible Causes	Check and/or Remedy
Cooling rate too slow	Ambient temperature is too warm	Move instrument to well-ventilated location.
Cycling time too long	Peltier failure.	Run cycle test diagnostic.
Instrument making too much noise	Fan failure.	Check for vent obstructions

RELATED DOCUMENTS AND RECORDS

6. Instruction Manual
7. Instrument Usage Log
8. Calibration and Maintenance Log
9. Warranty Card
10. Amendment Log

ABBREVIATIONS

1. AGE: Agarose Gel Electrophoresis
2. D/W: Distilled Water
3. µL: Microliter
4. µL: Microliter
5. mL: Milliliter
6. PCR: Polymerase Chain Reaction, the technique used to amplify DNA

pH Meter

Department of Biotechnology		STANDARD OPERATING PROCEDURE pH Meter (Working and Calibration)
SOP number	SOP14	
Effective Date	01/09/2018	
Prepared by	Mr. Himanshu Pande Ms. Sakina Lokhandwala	
Approved by	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

To describe a basic procedure on the operation, calibration, and activation of pH electrode in pH meter

SCOPE

This SOP determines the procedure on the operation of a pH meter in the laboratory using a standardized procedure.

LOCATION

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PRINCIPLE

A pH meter is an instrument that measures the hydrogen-ion activity in a liquid-based solution that helps in determining the acidity and alkalinity of solution termed as pH. This instrument measures the difference in electrical potential between a pH electrode and a reference electrode. This difference in electrical potential determines the pH of the solution.

MATERIALS

1. pH meter .
2. Sample (water-based solution)

ENVIRONMENTAL AND SAFETY CONTROLS

1. Switch off the instrument when not in use.
2. Always wear gloves while using the instrument.
3. Do not allow any other liquid or substance to enter the solution whose pH is to be determined.
4. Do not clean the pH meter with any kind of liquid when in use.

PROCEDURE

1. The pH meter is calibrated at least once before use.
2. The standard buffers of pH 4.0, pH7.0, and pH 9.2 are used for calibration.
3. These standard buffers must be used as recommended by the manufacturer Sensorex, i.e., it must be used for one time and should be disposed of after calibration is finalized.
4. To calibrate, the probe tip is to be rinsed using distilled water/ deionized water and dry the tip using filter paper/tissue paper. Place the tip into pH 7.0 buffer solution and switch to READ mode.
5. Adjust the control knob to read pH 7.0 on display.
6. Switch back to STAND-BY mode.
7. Remove the electrode from pH 7.0 calibration buffer, wash it with distilled water and place the electrode in calibration buffer (pH 4.0 or pH 9.2 as per requirement).
8. Switch to READ mode.
9. Adjust the control knob to read the respective pH on display.
10. Confirm the calibration by reading the pH of standard pH 7.0 buffer for the second time.

TROUBLESHOOTING

Buffer reading	Possible Cause	Corrective Action
6.2-6.8 in all buffers	a) Cracked pH glass b) Stress crack	a) Replace electrode b) Contact Sensorex for Return Authorization
7.00 in all buffers	a) Bad connection b) Internal short circuit	a) Check/fix connection b) Contact Sensorex for Return Authorization

Buffers read close to expected value but the speed of response* is slow (>30 seconds)	a) Dirty electrode pH glass and/or reference junction b) Temperature too low	a) Clean electrode per instructions included with it when shipped b) Flat pH glass pH electrodes should be used at temperature >10C/50F Bulb pH should be used at temperature >0C/32F
Large offset in buffers**	a) Reference poisoned b) Ground loop****	a) Contact Sensorex about special references b) The ground solution for tank or line to known earth ground or buy Sensorex
Short span*** (Typically less than 70%)	a) Dirty pH glass or reference junction b) aged electrode	a) Clean electrode per electrode's care and use instructions b) Replace electrode (too old)
Unstable or drifting reading	Reference dirty or plugged	Clean electrode per electrode's care and use instructions (do not use abrasives to clean reference junctions!)

RELATED DOCUMENTS AND RECORDS

1) Instruction Manual
2) Instrument Usage Log.
3) Calibration and maintenance log.
4) Warranty Card.
5) Amendment Log

ROTARY SHAKER

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP15	**Rotary Shaker**
Effective Date	01/09/2018	
Prepared by	Ms. Riya Dubey	
Approved by	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

To provide a basic method for using a rotary shaker.

SCOPE

This SOP covers all the procedures involving the use of the rotary shaker.The goal of this SOP is to standardize how each task in the laboratory is performed by every student.

LOCATION

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PRINCIPLE

Rotary shaker works on the principle of oscillation. The term oscillation implies a regular change in magnitude and orientation about a central axis. The main platform rotates in a circular motion and as it rotates shaking of the components present in the flask takes place. Oscillatory moments lead to the mixing of the different kinds of materials added in the flask.

MATERIALS

1. The sample in a conical flask.
2. Rough cotton cloth.

ENVIRONMENTAL AND SAFETY CONTROLS

1. Sample containing hazardous chemicals must be handled properly.
2. Safety equipment such as glasses, laboratory coats, nitrile gloves, closed toe shoes, hot gloves should be used during operation.
3. Waste should be disposed of in a container
4. For disposal of hazardous waste EH&S designated waste satellite container should be used.

PROCEDURE

1. The main platform has 12 spring housings so 12 flasks can be accommodated on the shaker.
2. The power switch is located on the right side of the machine towards the front, and the operation is run entirely from the power switch by switching it ON/OFF.
3. The regulator present adjacent to the power switch is used to control the revolutions per minute
4. Given below is the step by step procedure on how to operate rotary shaker
    a. Pre-analysis checklist:
        i. Make sure all safety equipment are in its place, i.e., glasses, laboratory coats, nitrile gloves, closed toe shoes, hot gloves
        ii. Check the machine for any remaining sample from spillage that occurred during the previous operation.
        iii. Remove the dust or foreign objects from the platform using a soft towel or cloth.
        iv. Make sure the machine is plugged in.
    b. Loading the shaker:
        i. Put your sample material in a 250ml flask.
        ii. Gently press the container in one of the spring housing until it is securely in place.
    c. Shaker operation:
        i. Plug the instrument into the electrical socket.
        ii. Turn on the machine using the power switch located on the right side.
        iii. Once the machine is powered ON, pressing the START/STOP button will cause the shaking to STOP
        iv. The rpm regulator can be rotated from low to high speeds per the requirement.
    d. Machine shutdown:
        i. Make sure the machine has come to a complete STOP.
        ii. Remove the flasks carefully.
        iii. Turn OFF the power using the power switch.

HANDLE AND STORAGE

1. This machine operates under high speed.
2. The lid should be closed while in operation.
3. If the lid unexpectedly opens, turn OFF the machine using the power switch to stop the rotation.
4. Be sure the instrument is plugged into the electrical socket.
5. Spills are possible if the cotton is not plugged properly.
6. Refer the instruction manual for proper loading technique and while handling bio-oil and other such chemicals; safety glasses and closed toe shoes are required.

MAINTENANCE

1. Always disconnect power during maintenance.
2. Spills should be cleaned immediately.
3. Any foreign object can be removed from inside by wearing safety gloves depending upon the nature of the object.
4. The rotating platform and springs can be wiped with a cloth or non- abrasive conventional household cleaner, never use caustic cleaning agents such as harsh soaps, phosphoric acids, bleaching solutions or scrubbing powder.
5. Culture spillage can be cleaned with absolute alcohol using a cotton swab.
6. If in case flask breaks downs, the broken glass pieces should be removed and thrown away carefully and by appropriate method spilled material should be cleaned.
7. In the case of large spills, specialized spill-kit should be used.
8. Filter paper and tissue paper can also be used for cleaning purpose.

TROUBLESHOOTING

FAULTS	POSSIBLE CAUSE
If the lid unexpectedly opens	Loose cotton plug
If the rotation stops	Poor electricity supply
If spark is observed	Damage in the wire or motor
If revolution per minute does not get regulated	Damage in regulator' knob

RELATED DOCUMENTS AND RECORDS

Manuals, Installation document, Calibration and main log, Amendment log.

SDS-PAGE APPARATUS

DEPARTMENT OF BIOTECHNOLOGY		STANDARD OPERATING PROCEDURE SDS PAGE
SOP No.	SOP16	
Effective Date	01/09/2018	
Prepared By	Ms. Namrata Saraf	
Approved By	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE:

To describe the appropriate operating instructions to perform SDS PAGE analysis of the protein sample.

SCOPE:

This procedure is applicable for SDS PAGE which is installed in a biotechnology laboratory.

LOCATION:

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Kishinchand Chellaram College,
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PRINCIPLE:

SDS- PAGE has been used as a tool for the separation and characterization of proteins. Since many functional proteins are composed of two or more subunits. Individual polypeptides are separated by gel electrophoresis, In the presence of detergent SDS, which denatures the protein after electrophoresis. Proteins are commonly detected by treating with Coomassie brilliant blue or silver stain. However, the separated polypeptides in the gel can also be transferred to nitrocellulose membrane, and individual protein can never be detected by using specific antibodies. SDS is carried out with a discontinuous buffer system with various pH and ionic strength. SDS employees 2 types of gels.

1. Stacking gel.
2. Separating gel / Resolving gel.

RESPONSIBILITIES:

1. The person who performs the experiment.
2. The person who is in charge of the apparatus.

PRECAUTIONS AND ENVIRONMENT AND SAFETY CONTROL:

1. Acrylamide is a neurotoxin. Pair of gloves should be used for handling polyacrylamide gel.
2. Dispose of in the fixative hazardous waste bottle.
3. Wear a mask while weighing the samples.
4. Avoid any contact with these materials. Wash hands after working with the substance.
5. Personnel wears protective clothing to prevent skin contact from splashes and for proper cleanup practices.
6. The primary hazard is a spill from poor handling practice.
7. Always clear up the table and instruments after the use or if spilled as mentioned in the SOP.
8. Clear acrylamide spills spontaneously step by step, i.e., first by using dry cloth than by wet cloth and then discard them as biohazard waste.

MATERIALS:

1. Protein sample
2. Protein standard
3. Molecular ladder
4. Acrylamide
5. Bisacrylamide
6. Power supply for protein electrophoresis
7. Microkini SDS PAGE apparatus
8. SDS running buffer
9. SDS stacking gel
10. SDS sample buffer
11. Micropipette and tips
12. Boiling water bath
13. Staining trays
14. Rotary shaker
15. Gel documentation instrument
16. Fixative solution
17. Coomassie brilliant blue stain.

PROCEDURE:

1. Arranging The Apparatus:
    a. Assemble the glass plates on the clean surface.
    b. Place the spacers on both the sides of the longer plate and then place the shorter glass plate on top of it.

c. Put them into the casting frame and clamp them carefully.
d. Glass plates should be aligned properly from the bottom ends.
e. Place this frame on the casting stand.
f. Cast the gel.

2. Preparation Of Resolving Gel And Stacking Gel:
    a. Combine all the reagents, i.e., SDS, 30% acrylamide/Bisacrylamide and/W except APS and TEMED.
    b. Add APS and TEMED to the solution just before pouring it in between the glass plates.
    c. Mix well by gently swirling.
    d. Allow the gel to polymerize for 15- 20 mins.
    e. Pour a small amount of isopropanol above the resolving gel to inhibit oxidation.
    f. Prepare the stacking gel solution by combining all the reagents except APS and TEMED.
    g. Drain the isopropanol with strips of filter paper.
    h. Add APS and TEMED to the solution and add this stacking gel in between the glass plates above the resolving gel.
    i. Place a comb in the stacking gel.
    j. Allow it to polymerize for 5- 10 mins.
3. Sample Preparation:
    a. Mix the protein sample with the sample buffer and heat the sample at 30℃ for 30 mins in a water bath.
4. For Running The Gel:
    a. Fill the inner chamber of the apparatus with 10X SDS running buffer.
    b. Remove the comb carefully.
    c. Insert the loading tip to a few mm from the well bottom and deliver the samples into the well.
    d. The power supply is connected and close the lid.
    e. Set the voltage up to 150V and run for 1 hour.
5. Staining the Gel:
    a. After running the apparatus, switch off the power supply and take out the gel plates and remove the gel.
    b. Place the gel in staining solution for 30 mins in shaker condition.
6. Destaining the Gel:
    a. Destain the gel the next day, by using destaining solution until the bands are properly seen and observe the bands
    b. Compare the bands with control.

TROUBLESHOOTING:

1. Eye damage due to splash
2. Power supply
3. Skin protection from flammable solvents.
4. Leakage of buffer

RELATED DOCUMENTS AND RECORDS:

1. Instruction manual.
2. Instrument usage log.
3. Calibration and maintenance log.
4. Warranty card.
5. Amendment log.

UV- TRANSILLUMINATOR

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP17	**Ultraviolet Transilluminator**
Effective Date	01/09/2018	
Prepared by	Mr. Umesh Pandey Ms. Sushma Jakhad	
Approved by	Dr. Sejal Rathod Ms. Sufiya Ansari	

PURPOSE

An Ultraviolet spectrophotometer is used to observe separated bands of nucleic acids after performing agarose gel electrophoresis. It works on the principle of fluorescence wherein the fluorescent dye used intercalates in the major grooves of nucleic acids. When this dye is exposed to UV light (under the UV spec), the dye fluoresces indicative of either DNA/RNA. DNA is observed as thick or thin, sharp or shabby sections through the gel, called as 'bands.'

SCOPE

UV Transilluminator is used in detections of different nucleic acids such as RNA, DNA (circular DNA, linear DNA, etc.) which have been separated by Agarose gel electrophoresis. Further, this nucleic acid can be used in sequencing, DNA fingerprinting, and other molecular biology techniques.A UV transilluminator can also be used to determine fluorescent pigments produced by certain types of bacteria.

LOCATION

3rd Floor, Biotechnology Laboratory,
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PRINCIPLE

UV transilluminator is an instrument commonly used in laboratories to detect fluorescent dye or micro-organism.Visualization of a fluorescent substance depends upon the wavelength

and optical filter used by the UV light.UV transilluminator emits UV light which causes the dye or micro-organism to fluoresce and become visible.

MATERIALS

1. Gel to be observed
2. Laser cut Acrylic parts.
3. UV bulb , filter , knob (of low and high intensity)and other hardware (foodie, digikey , TAP plastics).
4. AC power to Ballast.
5. Enclosure.
6. Cover lid.
7. Hinged safety lid.

ENVIRONMENTAL AND SAFETY CONTROLS

1. Exposure to UV light for a long duration can cause damage to human tissue such as a cataract in eyes and skin cancer.
2. UV light cause mutation in the nucleic acid or protein if kept exposing to UV light for long which cannot be used further for experiments.

PROCEDURE

1. Clean the bench and place UV transilluminator on it.
2. Ensure that all the switches and buttons are off and the cover is enclosed.
3. Wear appropriate gloves and lab coat to avoid contact with UV light.
4. Connect the power cord to the UV transilluminator and then switch on to check the UV light and after checking then off the UV light.
5. Place your sample on the surface of UV light glass and ON the UV light.
6. You can set the UV light on high or low intensity using the button as per the need.
7. Examine your sample and take photographs for future reference and then switch off the UV light and main pluck.
8. Clean the filter surface with soft cloth.
9. Keep the UV transilluminator back on safe place.

TROUBLESHOOTING

1. Mutation can occur if nucleic acids and proteins are kept expose for a long duration.
2. Power failure or not The illuminating then check the power connection and the power cord for proper connection.
3. Photobleaching of fluorophors upon exposure to light can become a significant problem, particularly when the experimental protocol is prolonged.

CALIBRATION

1. Instrument calibration is one of the primary processes used to maintain instrument accuracy.
2. The UV bulb, filters, and knobs should regularly calibrate at a fixed period of time to eliminating or minimizing inaccurate measurements.
3. Last calibrated date:_____

RELATED DOCUMENTS AND RECORDS

1. Catalogue number: UVT- 170601.
2. Serial number:UVT11201151.
3. Power :220V/50Hz ,300VA .
4. Warranty card
5. Calibration and maintenance log
6. Instrument usage log
7. Amendment log
8. BioEra Life Science Pvt. Ltd.

ABBREVATIONS

1. UVT = Ultra-violet transilluminators.
2. V = voltage.

UV-VISIBLE SPECTROPHOTOMETER

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP18	**UV-VISIBLE SPECTROPHOTOMETER**
Effective Date	01/09/2018	
Prepared by	Ms. Samrudhi K Pingle	
Approved by	Dr. SejalRathod Ms. Sufiya Ansari	

PURPOSE

To describe the procedure for operating the UV-VIS Spectrophotometer in the Biotechnology laboratory at Kishinchand Chellaram College and, to provide guidelines for its operation.

SCOPE

This procedure is applicable for operating the UV-VIS (Ultraviolet-Visible) Spectrophotometer in the Biotechnology laboratory at Kishinchand Chellaram College.

LOCATION

3rd Floor, Biotechnology Laboratory,
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PRINCIPLE

UV-VIS spectroscopy is a type of absorption spectroscopy in which light of ultra-violet region (200-400 nm) or of the visible light region (400-700 nm) is absorbed by the molecule.UV-VIS spectroscopy obeys the Beer-Lambert law, which states that *"when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with a thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution."*

The expression of Beer-Lambert law is-

$A = \log (I0/I) = \varepsilon CL$

Where A = absorbance

I0 = intensity of light incident upon sample cell

I = intensity of light leaving sample cell

C = molar concentration of solute
L = length of sample cell (cm)
Ɛ = molar absorptivity

From the Beer-Lambert law, it is clear that the greater the number of molecules capable of absorbing light of a given wavelength, the greater the extent of light absorption. This is the basic principle of UV-VIS spectroscopy.
The components of a UV-VIS Spectrophotometer are-
1. Light Source
2. Monochromator
3. Sample and Reference Cells
4. Detector
5. Amplifier
6. Recording Device

MATERIALS

1. Eye protection- Safety glasses
2. Protective clothing- Lab coat, Nitrile gloves
3. Glass cuvettes (Visible spectrophotometry)/ Quartz cuvettes (Ultraviolet spectrophotometry)
4. Tissue paper

ENVIRONMENTAL AND SAFETY CONTROLS

1. Dispose of solvent blank and sample solution in an appropriate waste container(s).
2. Solvents and samples could be hazardous or toxic. Hence, they must be handled with care. On spillage, the area must be cleaned and decontaminated properly before proceeding with the protocol.
3. Cuvettes could break and provide as sharp objects. In case if the cuvette breaks, the pieces must be carefully collected and safely discarded before continuing with further steps of analysis.

PROCEDURE

1. Ensure that the area is clean.
2. Switch on the main power.
3. Switch on the instrument.
4. Allow stabilizing for 15 minutes.
5. Set the desired wavelength with the help of buttons provided.
6. Put the mode selector at %T position.
7. The clean outer surface of cuvettes containing the blank and test sample(s) with tissue paper.
8. Insert the 2/3 filled blank and test sample(s) in the sample holders provided.
9. Cover the cuvette compartment.
10. Adjust the sample holder rod such that the monochromatic light passes through the blank.
11. Adjust to 0% transmittance with the help of blank (set zero control) by using % knob.
12. Adjust the sample holder rod such that the monochromatic light passes through the test sample whose Absorbance or % T is to be estimated.

13. Select the mode to Absorbance or % T using the mode selector.
14. The reading shown in the data position is the Absorbance or % T of the test sample.
15. Record the results and also make an entry in the instrument usage log book.
16. Switch off the instrument.
17. Switch off the main power.
18. Remove the cuvettes and wash with purified water or methanol as required.

TROUBLESHOOTING

PROBLEM	**POSSIBLE CAUSE**	**SOLUTION**
Stray light interference with monochromatic light causes apparent negative deviations from Beer's law.	Stray light may originate from imperfections in the dispersing element or in other optical surfaces, from optical aberrations or from damaged or worn components. Care must also be taken to eliminate extraneous light, e.g., light that leaks at the cell compartment or any mechanical boundaries. Stray light will cause apparent negative deviations from Beer's law	Ensure that the cuvette compartment is properly covered.
Beer's law plot for one component of a multi-component mixture may not be possible	More than one constituent of test sample absorbs at the wavelength of interest	Extract and purify the component to be estimated and then carry out the spectrophotometry.
Inaccurate measurements of blank and test sample	Cuvettes may be scratched or contaminated, due to fingerprints or condensation	Handle the respective glass or quartz cuvettes with care. Clean the cuvette surface with tissue paper every time before inserting it in the sample holder.
Inaccurate measurements of the test sample	The sample is impure and may contain contaminants	Using pure and known concentrations (standards) of the sample to be estimated to compare results obtained from the estimation of the test sample.

RELATED DOCUMENTS AND RECORDS

1. Instruction Manual
2. Instrument Usage Log
3. Calibration and Maintenance Log
4. Warranty Card
5. Amendment Log

VORTEX

Department of Biotechnology		STANDARD OPERATING PROCEDURE
		VORTEX
SOP number	SOP19	
Effective Date	01/09/2018	
Prepared by	Ms.Divya Goswami	
Approved by	Dr. SejalRathod	
	Ms. Hajra AS Gupta	

PURPOSE

To study the procedure for operation and cleaning of Vortex Mixer.

SCOPE

This will be useful in laboratories for effective mixing of the sample to create an even and homogenous solution.

LOCATION

3rd Floor, BSc Biotechnology Laboratory, Kishinchand Chellaram College, Vidyasagar Principal, K. M Kundnani Chowk, D. W. road, Churchgate, Mumbai - 400020

PRINCIPLE

A vortex mixer is a machine used to mix liquids thoroughly. It contains an electric motor with the drive shaft oriented vertically which is attached to a rubber piece which is in a cup shapes and is mounted slightly off-center.

The motor moves the cup, and it moves rapidly

in a circular motion. When the containers are kept on the cup the liquid inside, and a vortex moves The speed can be set according to the requirement.

MATERIAL

- Test tube with suspension
- Vortex machine

ENVIRONMENTAL AND SAFETY CONTROLS

Proper machine guarding should be taken:

It protects users from dangers such as rotating parts, flying chips, and sparks. This is to protect the workplace safety dangers of machinery.

Do not disturb the moving machinery:

Do not disturb the person operating it or the running machine. Using such machines required a lot of focus that is broken if the user is distracted. Touching a moving part of machinery may damage the machine and can also cause damage to the user.

Never Use Machinery That You Are Not Trained For:

Machinery requires training so that it can be operated properly. People are trained through

several procedures by a person who already an expert in handling the machine. Operating machinery without training can lead to serious risk for injuries.

– DO NOT play with the working machine.

– Switch off the machine before cleaning.

– Cleaning should be done as per the instructions.

PROCEDURE

- Place the suspension tube in the cup.
- Turn on the switch; the vessel should be kept properly on the cup before switching on the plug.
- Turn on speed knob to the speed; the mixer will run according to this setting.
- Vary the speed so as to mix properly.
- Switch “OFF” the machine when the required mixture is obtained.

TROUBLESHOOTING

WARNING! DO NOT use the Vortex machine for which it is not designed; the user should know that the protection which has been provided us with no use if the instrument's instructions are not being followed properly.

The machine should always be used on a level surface for better results and safety.

DO NOT lift the Vortex machine from its head; they can break off if done so.

To avoid electric shocks disconnecting the power is the best option.

DO NOT use the machine if it shows signs for electrical or mechanical damage.

Problem - Machine will not run. **Cause -** Mechanical or motor barrier

Solution – Remove the barriers and check several times so that it is confirmed that no barrier is present.

Problem – Machine makes an excessive unwanted sound. **Cause -** Error in Sensor fan or Motor position.

Solution - Cannot be repaired by the user.

RELATED DOCUMENTS AND

RECORDS

- Instruction Manual
- Instrument usage log
- Warranty card
- Amendment log

CALIBRATION

A suspension tube is prepared.

The tube is fixed at an angle on the cup, and then the cup is moved by a motor at some rotation frequency.

WATER BATH

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP20	
Effective Date	01/09/2018	**Water Bath**
Prepared by	Mr. ShivamBisht	
Approved by	Dr. SejalRathod Ms. Hajra AS Gupta	

PURPOSE

It is used to warm or heat samples in water at a constant temperature over a long period of time.

SCOPE

This procedure is applicable for operation and calibration of water bath which is installed in the Biotechnology laboratory. It is used to maintain the sample or reaction mix at a set temperature.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. KundnaniChowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

A water bath is a device that maintains water at a constant temperature. It allows the heating of small amounts of components over a period of time without changing the concentration of constituents by evaporation. It converts electrical energy to heat energy.

MATERIALS

1. Motor
2. Magnet
3. Heater
4. Stirrer inside
5. Reagent stirrer

ENVIRONMENTAL AND SAFETY CONTROLS

1. Do not operate without water.
2. Switch OFF when the instrument is not in use.
3. Clean from inside and change the water daily.
4. Keep the lid closed when not in use to prevent evaporation

PROCEDURE

1. Ensure that the instrument is clean and calibration of temperature indicator is within due date of calibration.
2. Fill and check the water level, if required fill purified water to the acceptance level .The minimum water level is indicated by a black line on the water level indicator on the left.
3. Switch ON the ring both by pressing ON/OFF switch.
4. The digital temperature controller cum indicator will indicate the actual temperature of the water.
5. Set the desired temperature by pressing the PRESS to SET switch and adjusting the SET pot.

TROUBLESHOOTING

1. The procedure involves working with high voltage that can cause injury and death so the instrument should be handled with care under supervision.
2. If the water bath feels faulty, don't use the instrument and immediately switch OFF the instrument.

CALIBRATION

1. Instrument calibration is one of the primary processes used to maintain instrument accuracy.
2. The timer set for water bath and temperature should be adjusted properly as per requirement and checked in regular intervals.

RELATED DOCUMENTS AND RECORDS

1. Calibrated on:28/7/18
2. Serial number:NA11ED041
3. Cert no. :TCM79
4. Warranty card
5. Calibration and maintenance log
6. Instrument usage log
7. Amendment log

WEIGHING BALANCE

Department of Biotechnology		STANDARD OPERATING PROCEDURE
SOP number	SOP21	**WEIGHING BALANCE**
Effective Date	01/09/2018	
Prepared by	Ms. Sejal Jain	
Approved by	Dr. SejalRathod Ms. Sufiya Ansari	

PURPOSE

To provide a basic method for using a weighing balance.

SCOPE

This SOP covers all the procedures involving the use of the weighing balance.The goal of this SOP is to standardize how each task in the laboratory is performed by every student.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. KundnaniChowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

The working principle of a weighing balance is “Magnetic Force Restoration.” One end of an object made of aluminum is fixed, and the sample is placed on the other end. The weight of the sample causes the elastic body to bend, which causes strain in the gauges attached to the elastic body, which changes the output of electricity. The mass is then obtained from that amount of electricity.

MATERIALS

1. Dye cast aluminum is used for long-term stability & accurate results.
2. Different weighing units like gm, mg, etc. are used.
3. The large backlit LCD display for easy reading.
4. A hanger for below balance weighing.
5. Fully automatic internal calibration with built-in weight.

6. Selectable stability & filter level false draft shield interior.
7. Improved repeatability for better.

ENVIRONMENTAL AND SAFETY CONTROLS

1. The location and environment in which the weighing balance is located.
2. It is recommended that mass calibration labs have a small antechamber to prevent the ingress of dust.
3. If unacceptable drift in weighing results is to be avoided the control system should not induce rapid fluctuations in temperature.

PROCEDURE

BALANCE ENVIRONMENT:-

1. Check balance level on the indicator.
2. Check balance for cleanliness.
3. Clean the balance with a soft brush.

BALANCE CALIBRATION:-

1. Before measuring make sure the balance is calibrated.
2. Recalibrate the weighing balance if the power supply fails.
3. All the connection of the balance must be proper, and the balance is on.
4. Balance must be kept on a shockproof bench top.
5. If the balance is showing some reading on display, then press the key.
6. The door must be opened from one side & keep the paper and close the door.
7. Then open the door and measure the required sample.
8. Keep adding the sample till you get desired weight on display & close the door.
9. After getting the desired weight, open the door & take out the sample the sampling paper.
10. Close the door & press tare key again.
11. Balance should be 0.000.

MAINTENANCE

1. The most important thing in lab balance maintenance is to keep the balance clean and properly maintained.
2. It also depends upon how often you use your balance, the working environment, and any other regulatory mandates in your lab.
3. It’s best to remove the pan when cleaning or storing your balance.

PRECAUTIONS

1. Not remembering to calibrate your balance is another fault that can lead to large problems, but is easily solved by maintaining a record of maintenance tasks.
2. A maintenance record is “very beneficial, especially if there are multiple users operating the balance.

3. Rough handling of a balance is one of the major factors to a balance not functioning at proper levels.
4. Make use of a soft brush for cleaning.
5. Remove pan carefully if required.
6. Clean with a slightly wet cloth.
7. Never touch the weighing pan while performing.

RELATED DOCUMENTS AND RECORDS

1. Instruction Manual
2. Installation document
3. Calibration and maintenance log
4. Warranty Card
5. Amendment log

ABBREVIATIONS

1. gm-grams
2. mg-milligrams
3. SOP-Standard Operation Procedure

REFERENCE

1. Manual
2. Books
3. Journals

WESTERN BLOT APPARATUS

Department of Biotechnology		STANDARD OPERATING PROCEDURE Western Blot Apparatus
SOP number	SOP22	
Effective Date	01/09/2018	
Prepared by	Mr. Shubhankar Dube & Mr. Gaurav Kudale	
Approved by	Dr. Sejal Rathod	
	Ms. Sufiya Ansari	

PURPOSE

The purpose of this document is to describe the procedure for performing Western Blot analysis for the characterization of proteins.

SCOPE

This procedure may be used for the detection of proteins with molecular weights in the range of 10KDa to 250KDa. Other procedures must be used for proteins outside of this range.

LOCATION

3rd Floor, Biotechnology Laboratory,
Kishinchand Chellaram College,
Vidyasagar Principal K. M. Kundnani Chowk,
D. W. Road,
Churchgate,
Mumbai-400 020

PRINCIPLE

The western blotting technique is used for identification of a particular protein from the mixture of protein. In this method labeled antibody against a particular protein is used to identify the desired protein, so it is a specific test. Western blotting is also known as immunoblotting because it uses antibodies to detect the protein.

MATERIALS

1. Transfer Buffer
2. 10X Blocking Buffer (note 1)
3. TBST (note 2)

4. TBS (note 3)
5. Appropriate Primary and Secondary Antibodies
6. SigmaFast Developer Tablets (cat# B-5655) or CN/DAB Substrate Kit (cat# 34000, Pierce)
7. Milli-Q Water
8. Whatman #1 Filter Paper
9. Nitrocellulose Membrane
10. Blotting Cassette, complete with foam pads
11. Blotting Chamber
12. Blotting Container
13. Shaker Table
14. Power Supply

PROCEDURE

1. Perform SDS PAGE analysis with the desired protein sample.
2. Cut proper sized pieces of Whatman filter no.1 paper and appropriate sized nitrocellulose membrane while the gel is running.
3. On top of the foam inserts on the grey side of the cassette, place 1-2 sheets of filter paper and place membrane over it.
4. Close the cassette and place into the blotting chamber.
5. The blotting chamber is now filled with transfer buffer to the marked line on the side.
6. Let it soak in the transfer buffer until the membrane is completely wet.
7. After SDS PAGE is completed, open the cassette in such a way that the grey side acts as a base for the membrane.
8. Dip the gel into the transfer buffer after removing the gel from the glass plate.
9. Place the SDS-PAGE gel on top of the membrane with the marker on the left side of the membrane.
10. Wet the gel with a small amount of transfer buffer from the chamber.
11. Make sure that there are no air pockets formed between the gel and the membrane, remove those if there are any.
12. Place filter papers over the gel from the black side of the cassette and close the cassette.
13. Place the cassette back into the blotting chamber and put the lid on it.
14. Make sure the positive lead is on the side of the grey cassette in the chamber so that the current is driven towards the grey side and force proteins onto the membrane.
15. Plug the leads into a power supply and set the voltage to 50V for 1 hour, or 5-10V for 15 hours for an overnight transfer.
16. Once the transfer is complete, turn off the power supply and remove the cassette and open.
17. Prepare 1X blocking buffer by making a 1:10 dilution of the 10X blocking buffer in a small blotting container (note 5).
18. Once the transfer is complete, the gel is discarded along with filter paper while the membrane is moved into a small blotting container.
19. Place blotting container on a shaker table and let it incubate for at least an hour (note 6).

20. Discard the blocking buffer and rinse the membrane 3 times for a short duration with TBST and then keep it on a shaker table for 5 minutes.
21. While the membrane is rinsing, dilute the primary antibody to the proper titer in TBST.
22. Incubate the membrane with primary antibody for at least an hour.
23. Discard primary antibody (note 7), rinse the membrane three times for short duration with TBST, then keep it on a shaker table for 5 minutes.
24. Now, apply the alkaline phosphatase conjugated secondary antibody diluted to the proper titer in TBS (note 8).
25. Incubate with secondary antibody for 30-45 minutes; however, care must be taken that it isn't left at incubation for long as it may increase the non-specific binding.
26. During incubation, place a SigmaFast developer tablet in 10ml of Milli-Q water. Vortex to dissolve it.
27. If CN/DAB kit is to be used, bring the kit to room temperature and make a 1X solution of CN/DAB substrate in stable peroxidase substrate buffer. Filter the solution just prior to development through a 0.2µm filter.
28. After incubation with the secondary antibody, rinse the membrane three times shortly with TBS, then keep it on a shaker table for 5 minutes.
29. Apply the appropriate developer solution to the membrane and wait for the blot to develop.
30. Once the band of interest appears, discard the developing solution and rinse with Milli-Q water several times.
31. Place the blot on a paper towel and fold the towel to cover the blot.
32. Once dry, the blot can be placed in a notebook or scanned.

Notes:

1. 10X blocking buffer is 10% BSA in TBST.
2. Composition of TBST:- 1.21g Tris, 8.77g NaCl pH7.4 2.5ml 20% Tween 80 or 0.5ml Tween 80 (1L)
3. Composition of TBS:- 1.21g Tris, 8.77g NaCl, pH7.4 and QS to 1L water.
4. Handle the membrane with gloves or tweezers; even those, however, might cause the development of some undesirable background.
5. Generally, 1-3% BSA is used to block. A minimum of 10 ml of blocking buffer is required for a small blocking container.
6. The membrane can also be in blocking buffer overnight at 4^{o}C.
7. Most primary antibodies can be used more than once, save it in a labeled falcon tube and store at -20^{o}C.
8. Two types of secondary antibodies are used the most:
    - Alkaline phosphatase conjugated- use SigmaFast Tab to develop.
    - Peroxidase conjugate-use CN/DAB Substrate Kit to develop

TROUBLESHOOTING

- No signal
    1. The primary antibody and the secondary antibody are not compatible:
       Use a secondary antibody that was raised against the species in which the

primary was raised (e.g., if the primary is raised in rabbit, use an anti-rabbit secondary).

2. Not enough primary or secondary antibody is bound to the protein of interest:
   Use a higher concentration of antibody or incubate longer (e.g., overnight) at 4°C.
3. There is cross-reactivity between the blocking agent and the primary or secondary antibody:
   Use a mild detergent such as Tween 20 or switch blocking reagent (i.e., commonly used blocking reagents are milk, BSA, serum or gelatin).
4. The primary antibody does not recognize the protein in the species being tested:
   Check the datasheet or perform a BLASTp alignment to see whether your antibody should react with the target protein. Run the recommended positive control.
5. There is insufficient antigen:
   Load at least 20–30 µg protein per lane, use protease inhibitors and run the recommended positive control.
6. The protein of interest is not abundantly present in the tissue:
   Use an enrichment step to maximize the signal (e.g., prepare nuclear lysates for a nuclear protein).
7. There is a poor transfer of protein to membrane:
   Check the transfer with a reversible stain such as Ponceau S. If proteins have not transferred effectively, check the transfer was not performed in the wrong direction. If using PVDF membrane, make sure that you pre-soak the membrane in methanol and then in transfer buffer.
8. Excessive washing of the membrane:
   Reduce the number or duration of washing steps.
9. Overuse of the primary antibody:
   Use fresh antibody as the effective concentration is lowered upon each use.
10. The secondary antibody is inhibited by sodium azide:
    Do not use sodium azide together with HRP-conjugated antibodies.

The detection kit is old, and the substrate is inactive:

Use a fresh substrate

- High background
  1. Blocking of non-specific binding might be absent or insufficient:
     Increase the blocking incubation period and consider changing the blocking agent, we recommend 3–5% non-fat dry milk, BSA, or normal serum for 1 h at room temperature. These can be included in the antibody buffers as well.
  2. The primary antibody concentration may be too high:
     Titrate the antibody to the optimal concentration. Incubate for longer but in more dilute antibody (a slow but targeted binding is best).
  3. The incubation temperature may be too high:
     Incubate membrane at 4°C.
  4. The secondary antibody may be binding non-specifically or reacting with the blocking reagent:
     Run a secondary control without the primary antibody.

5. Cross-reactivity between the blocking agent and primary or secondary antibody:
   Add a mild detergent such as Tween 20 to the incubation and washing buffer.
6. For phospho-specific antibodies: milk contains casein which is a phosphor-protein: The phospho-specific antibody will detect casein present in the milk causing high background. Use BSA as a blocking reagent instead of milk.
7. The washing of unbound antibodies may be insufficient:
   Increase the number and time of washes.
8. Your choice of membrane may give high background:
   Nitrocellulose membranes are considered to give less background than PVDF.
9. The membrane has dried out:
   Care should be taken to prevent the membrane from drying out during incubation

- Multiple bands
  1. Cell lines that have been frequently passaged gradually accumulate differences in their protein expression profiles:
     Go back to the original non-passaged cell line and run these samples in parallel.
  2. The protein sample has multiple modified forms in vivo such as acetylation, methylation, myristylation, phosphorylation, glycosylation, etc.:
     Examine the literature and use an agent to remove modifications where possible so that the protein runs at the expected size.
  3. The target in your protein sample has been digested (more likely if the bands are of lower molecular weight):
     Make sure that you incorporate sufficient protease inhibitors in your sample buffer.
  4. Unreported novel proteins or different splice variants that share similar epitopes and could possibly be from the same protein family are being detected:
     Check the literature for other reports and also perform a BLAST search; use the cell line or tissue reported on the datasheet.
  5. Primary antibody concentration is too high:
     Try decreasing the concentration of the primary antibody. Run a secondary antibody control (without the primary).
  6. The antibody has not been purified:
     Try to use an affinity purified antibody. This will often remove non-specific bands.
  7. The bands may be non-specific:
     Where possible use blocking peptides to differentiate between specific and non-specific bands. Only specific bands should be blocked (and thus disappear).
  8. The protein target may form multimers:
     Try boiling in Laemmli buffer for 10 min rather than 5 min to disrupt multimers.

- Uneven white spots on the blot

  Air bubbles were trapped against the membrane during transfer, or the antibody is not evenly spread on the membrane:
  Make sure you remove bubbles when preparing the gel for transfer. Incubate the antibodies while agitating.

- Black dots on the blot
  The antibodies are binding to the blocking agent:
  Filter the blocking agent.

- White bands on a black dot (negative on expected blot)
  Too much primary and/or too much secondary antibody:
  Dilute the antibodies more.
- Molecular weight marker lane is black
  The antibody is reacting with the molecular weight marker:
  Add a blank lane between the molecular weight marker and the first sample lane.

- Band of interest is very low/high on the blot
  Separation is not efficient:
  Change the gel percentage: use a higher percentage for small proteins and a lower percentage for large proteins.

- Smile effect on the bands
  1. Migration was too fast:
     Decrease the voltage while running the gel.
  2. Migration was too hot (changing the pH and altering the migration):
     Run the gel in the cold room or on ice.

- Uneven band size in lanes probed for the same protein
  Gel has set too quickly while casting, and the acrylamide percentage is not even throughout the gel:
  Review the recipe of the gel and the addition of TEMED to the gels, add some 0.1% SDS in water to the top of the migrating gel while it sets to stop it from drying.
- Uneven staining of the gel
  1. Contamination from bacteria:
     Keep antibodies at 4°C and use fresh buffers covering the gel.
  2. Not enough antibody volume:
     Make sure the membrane is covered with the antibody and incubate while agitating.

RESPONSIBILITY

- General handling procedures
  1. Do not handle membranes with fingers; use forceps.
  2. Set up blotting sandwich
     tight to avoid streaking.
- Do's and Don'ts
  1. Use HQ filter paper during transfer to avoid artifacts.
  2. Use powder-free gloves.
- No contaminations & autofluorescent substances
  1. CoomassieTM
  2. TritonTM X-100

3. BFB
4. Polyacrylamide gel fragments.

- Do not write on the membrane with a pen, use pencil/cut corners.

RELATED DOCUMENTS AND RECORDS

6. Instruction Manual
7. Instrument Usage Log
8. Calibration and Maintenance Log
9. Warranty Card
10. Amendment Log

ABBREVATIONS

1. SDS-PAGE – Sodium dodecyl sulphate polyacrylamide gel electrophoresis
2. TBST – Tris-buffered saline with Tween 20
3. TBS – Tris buffered saline
4. BLAST – Basic Local Alignment Search Tool
5. HRP – Horse radish peroxide
6. BSA – Bovine serum albumin
7. PVDF – Polyvinylidene difluoride

ABOUT THIS MANUAL

The purpose of this SOP manual is to provide a detailed step-by-step guideline of working of all equipments housed by the Biotechnology Laboratory of KC College. This SOP manual is an effort brought together *By the Students* and *For the Students*. The objective of this manual is to avail defined and clear instructions on how to operate an instrument to any incoming individual with a research question. The authors have taken the initiative to restate every detail recorded in the catalog and expand on the existing functional and safety measures to put together a solely sufficient SOP manual. The editors have ensured that the manual conveys accurate information which is of significant relevance; while also encouraging the use of reader friendly language. The manual provides a purpose and scope for every basic and advanced device installed in the laboratory with simple and elaborate operating procedures. The authors and editors have also included necessary environmental and safety controls along with a section on troubleshooting for every apparatus. This piece of instructive science writing will benefit all the future incoming students and scholars for their contribution to science and society.

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