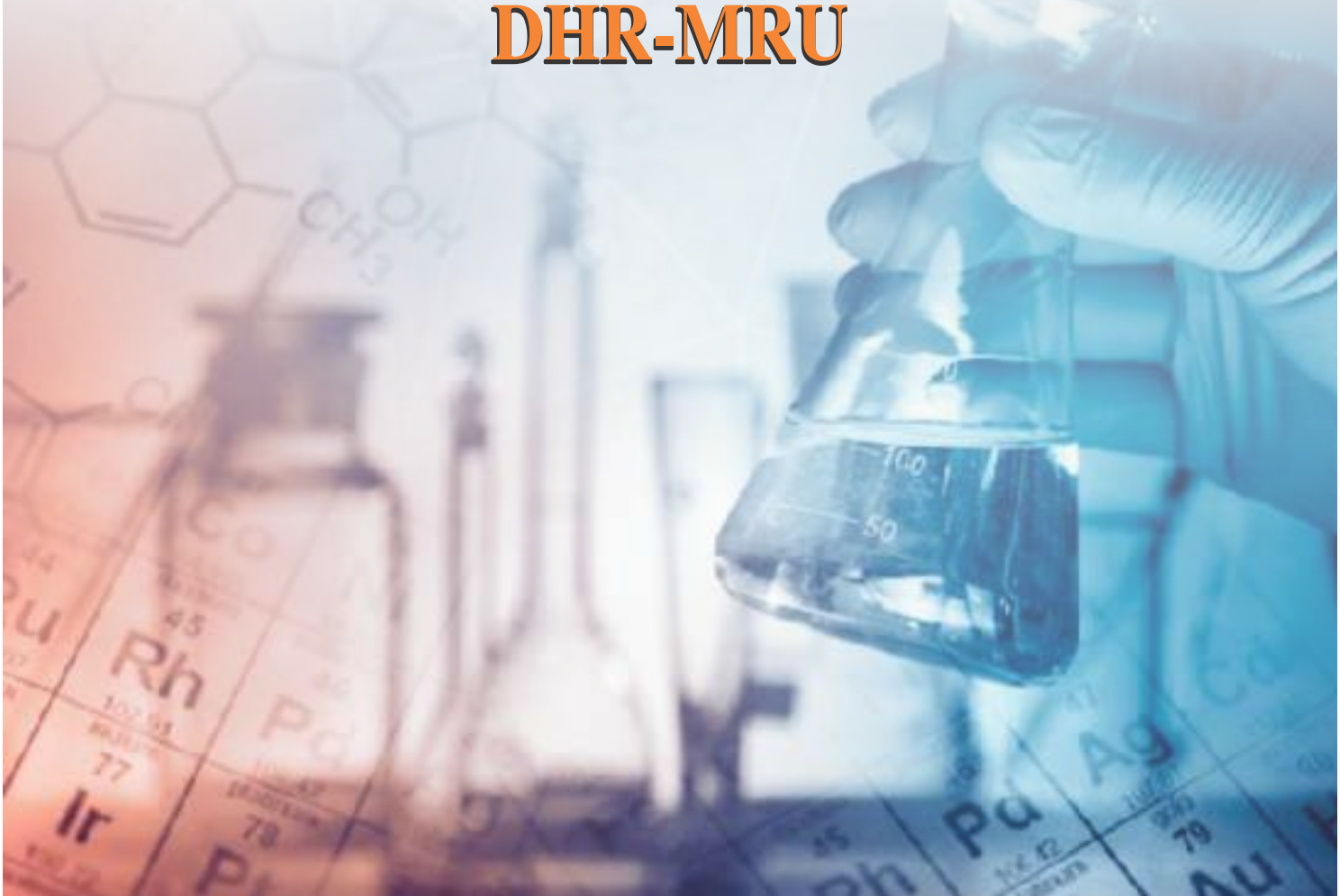
King George's Medical University Lucknow

Handbook of Standard operating procedures & laboratory usage in DHR-MRU



Brief Introduction

KG Medical University has established running Multi-Disciplinary Research Laboratory in its campus in year 2017. The lab is sponsored by Department of Health Research, Govt of India with a budget of 5.25 crore on five year term. After five years the state government will take over the facility. The lab has a manpower of two research scientist, 2 lab technicians and 1 data entry operator. The lab is first of its kind in UP and is aimed at promoting basic, clinical and translational patient oriented research. It has genetics, genomics, proteomics, biobanking and cell culture facility. The facility is open to researchers and clinicians from all KGMU affiliated colleges and other collaborating institutes. Main objectives of the lab are to encourage and strengthen an environment of research in medical colleges, to bridge the gap in the infrastructure which is inhibiting health research in the Medical Colleges by assisting them to establish multidisciplinary research facilities with a view to improving the health research and health services, to ensure the geographical spread of health research infrastructure, in order to cover un-served and under-served Medical Colleges and other institutions and to improve the overall health status of the population by creating evidence-based application of diagnostic procedures/processes/methods. The lab will also train young researchers on various molecular biology techniques and principles involved in human. The facility will act like innovation incubator facilitating conversion of research ideas to industry oriented outputs useful in public health research like drug development.

Facilities Available

1. Dedicated labs:

1) Basic Genetics & Elisa Lab
2) PCR Lab
3) Genomics Lab
4) Flowcytometry Lab

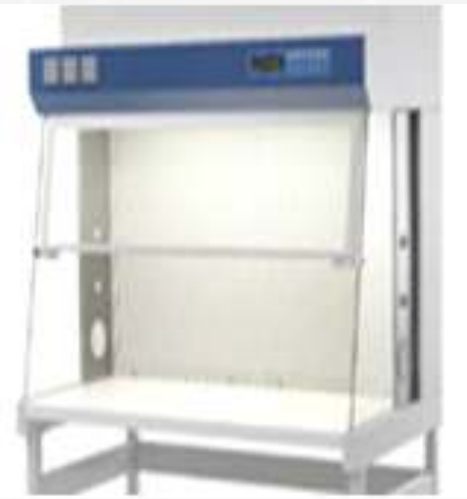
5) Cell Culture Lab
6) Biobanking Room

2. Instruments:

1) PCR
2) Gel Doc System
3) Centrifuge- Refrigerated and non refrigerated
4) Table Spin
5) Nanodrop
6) Autoclave
7) ELISA reader
8) Electronic weigher
9) Shaker
10) Incubator
11) Vertex
12) Shaker
13) Laminar Flow Hood
14) Mili Q water System
15) Electronic pH Meter
16) Multiplate washer
17) Pippettes

Biosafety Cabinet

A biosafety cabinet (BSC)—also called a biological safety cabinet or microbiological safety cabinet—is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) pathogens requiring a defined biosafety level. The primary purpose of a BSC is to serve as a means to protect the laboratory worker and the surrounding environment from pathogen.



SOP for Usage & Maintenance

- Please ensure that the equipment is placed in a suitable environment with proper ventilation, dust & draught free & preferably in an Air-conditioned room for optimal performance.
- Please ensure that the equipment is connected to an appropriate Voltage Stabilizer for proper functioning.
- Please ensure that the workbench is properly cleaned/ disinfected after every use or if any spillage is observed.
- Please ensure that the openings in the front & back on the work bench should not be blocked during the usage of the workbench.
- Please ensure that the front glass hood is kept in safe working position during usage of the work bench else an audible alarm will be indicated.
- Please ensure that depending on the hazard level of the agents involved, the operator must prepare appropriate decontamination procedures for the device & accessories used in the workbench.
- Please ensure that the gas supply to the safety burner is closed properly after every usage.

Please DO NOT

- Use the equipment if the alarm is indicated continuously for a failure condition & if the cause for the failure is not rectified.
- Work with or store substances in the Safety cabinet, which may cause reaction with other substances or may help in formation of toxic gases.
- Work with or store substances in the Safety cabinet, which may form combustible or explosive mixtures in combination with air.

- Please note that all users of the workbench have to go through & refer to the Instruction manual for proper operation & usage of the equipment.
- If you find any abnormal operation, please contact Thermo Fisher Scientific Toll Free OR our office.

CO2 INCUBATOR

A cell culture incubator is designed to maintain a constant temperature and high humidity for the growth of tissue culture cells under a CO2 atmosphere. Typical temperature settings range from 4C to 50C, and CO2 concentrations run from 0.3 to 19.9%. Non-corrosive stainless steel interiors are standard, but some newer models feature antimicrobial copper surfaces to prevent contamination. Auto decontamination is done using heat or UV light.

SOP for Usage & Maintenance

- Please ensure that the equipment is placed in a suitable environment with proper ventilation, dust free & preferably in an Air-conditioned room for optimal performance.
- Please ensure that the equipment is connected to a delayed-start 5 KVA Voltage Stabilizer for proper functioning. The stabilizer should be a servo control voltage stabilizer.
- Use caution when handling the filter. The media can be damaged if it is mishandled. To avoid damage to the incubator, do not operate the unit without the HEPA filter in place.
- Connect the incubator to a grounded dedicated circuit only.

The power cord connector is the mains disconnect device for the incubator. Position the unit so that it can be easily disconnected.

- Sterilized distilled, demineralized or deionized water used in the humidity pan must be within a water quality resistance range of 50K to 1M Ohm/cm or a conductivity range of 20.0 to 1.0 uS/cm. to protect and prolong the life of the stainless steel. Use of water outside the specified range will decrease the operating life of the unit and void the warranty.
- High concentrations of CO2 gas can cause asphyxiation! OSHA Standards specify that employee exposure to carbon dioxide in any eight-hour shift of a 40-hour work week shall not exceed the eight-hour time weighted average of 5000 PPM (0.5% CO2). The short term exposure limit for 15 minutes or less is 30,000 PPM (3% CO2). Carbon dioxide monitors are recommended for confined areas where concentrations of carbon dioxide gas can accumulate.
- This incubator is designed to be operated with CO2 gas only. Connecting a flammable or toxic gas can result in a hazardous condition.
- The CO2 gas supply being connected should be industrial grade 99.5% pure and should not contain siphon tubes.
- Install a two-stage pressure regulator at the cylinder outlet. The high pressure gauge at the tank should have 0-2000 psig range and the low pressure gauge, at the incubator inlet, should have a 0-30 psig range. Input pressure to incubator must be maintained at 15 psig (103.4 kPa), ±5 psig.
- Before making an calibration or adjustments to the unit, it is imperative that all reference instruments be properly calibrated. To ensure accurate calibration, the unit will not

allow CO2 to be spanned below 3%. If the cabinet does not contain at least 3% CO2, increase the setpoint and allow the unit to stabilize before completing this procedure.

- ❖ Alcohol even a 70% solution, is volatile and flammable. Use it only in a well ventilated area that is free from open flame. If any component is cleaned with alcohol, do not expose the component to open flame or other possible hazard. Allow the alcohol to fully dry before turning power on.
- ❖ During abnormal operation (Electrical or Mechanical Failure), avoid opening the door, as this would increase the temperature of the freezer. Immediately contact your Maintenance department or our office.

Legend RT : Refrigerated Centrifuge

The Legend RT is a general-purpose tabletop centrifuge for biotechnological and pharmaceutic research that moves high capacity centrifugation onto the fast track. It can process nominally three liters of sample in a single run. There are various rotors available that can achieve high RCFs and accommodate a wide range of accessories for all common tube types, micro titer and deep well plates.

SOP for Usage & Maintenance

- ❖ Please ensure that the equipment is placed in a suitable environment with proper ventilation, dust free & preferably in an Air-conditioned room for optimal performance.
- ❖ Please ensure that the equipment is connected to a delayed-start 5 KVA Voltage Stabilizer for proper functioning.

- To ensure sufficient air circulation, a minimum distance from the wall of 10cm (4 inches) at the back and of 15cm (6 inches) on each side must be kept.
- The centrifuge must be protected from heat and direct sunlight.
- Use caution when touching rotors as they may be hot after long runs at high speed.
- All positions have to be loaded with identical carrier buckets always. Always make sure the rotor is uniformly loaded.
- The rotor has to be retired upon reaching the end of its life, which depends on the number of runs that may be performed, and varies depending on run speed.
- The rotor and accessories must be clean and inspected regularly : do not use when showing signs of corrosion or cracking.
- Before use, the seals in the rotors and rotor covers, as well as the aerosol tight caps, have to be checked for abrasion or damage and slightly greased. Replace damaged O-rings and seals.
- Replace damaged or clouded caps and lids of rotors and tubes immediately.
- The aerosol-tight bio-containment of air vessel rotor is only warranted in a horizontal position!
- Please mind the maximum permissible filling volume during centrifugation of dangerous samples.
- Regularly check the proper positioning of the rotor and re-tighten the collect chuck as needed.
- During a power failure it is impossible to lock the lid once the emergency lid release has been used. Never stop the rotor using your hands or tools.

- During cleaning liquids and especially organic solvents should not come into contact with the drive shaft and the ball bearing. Organic solvents may decompose the lubricant of the motor bearing.
- Please do NOT attempt to open the door during abnormal operation (Electrical or Mechanical) as this would be against Safety of the user & environment. Please contact Thermo Fisher Scientific Service immediately.

Multiskan Ex

An ideal tool for use bench-top microplate photometer for basic ELISA applications. Other applications include use in Immunoassays, Protein assays , Growth curve and hormone assays, Endotoxins , Food diagnostics, HIV assays, Hybridization assays, Minisequencing assays, Cytotoxicity, Cell adhesion , Signal transduction, Endotoxins, Anti oxidants, Food diagnostics

SOP for Usage & Maintenance

USAGE

- Plug into the mains & switch on. The filter wheel and the lamp is installed in the unit.
- Select the RS format for Printer or Computer.
- Select the plate movement to be continuous or stepwise.
- Select print out on the built-in printer if required.
- If built-in printer, then select printing width.
- If external printer, then select printout spacing.

- Select shaking, if required.
- Select the Program Module.
- Select the Measurement Mode – Measurement Parameters.
- Select the Calculation Mode – Calculation Parameters.
- Save the Program.
- Connect printer or computer.
- Check the BAUD Rate, Transmit/Receive pin Configuration & Handshake with DIL Switches.
- Press START key. Results are recorded on built-in printer, external printer or computer.

MAINTENANCE

- Make sure the working area is flat, dry, clean and vibration-proof and leave additional room for accessories, cables, reagent bottles, etc.
- Make sure the ambient air is clean and free of corrosive vapors, smoke and dust.
- Please ensure that the equipment is connected to a delayed-start 2 KVA Voltage Stabilizer for proper functioning.
- DO NOT operate the instrument in an environment where potentially damaging liquids or gases are present.]
- DO NOT touch or loosen any screws or parts other than those specially designated in the instructions. Doing so might cause misalignment and will invalidate the instrument warranty.
- DO NOT insert the computer's serial cable to the printer port. This may cause unexpected problems.
- Never spill fluids in or on the equipment.
- DO NOT touch the filters with your bare fingers. When

installing the filter wheel and the lamp, do not touch any other mechanical or electronic part.

- DO NOT touch the reflecting surface of the lamp or the bulb itself.
- Never operate your instrument from a power outlet that has no ground connection. Never use a mains supply cable other than the Thermo Scientific mains supply cable designed for your region. Make sure that the protective conductor inside or outside the instrument is never interrupted and that the protective conductor terminal is not disconnected.
- Operate the instrument only with software and hardware specifically designed or selected for it. The instrument does not verify the logic flow of the received commands. Thermo Fisher Scientific assumes no liability for the use of third-party software applications.
- The printer destination is set to external printer as default. If no printer is connected to the instrument, the message NO CENTRONICS PRINTER is displayed.
- Do not use the internal printer without paper. Make sure the paper will not roll between the paper roll and the instrument. Help the paper to pass over the paper roll with your finger, if necessary.
- Wipe the instrument surfaces with a soft cloth or tissue paper moistened with deionized distilled aqua, a mild detergent (SDS, sodium dodecyl sulfate) or soap solution after every use.
- Painted surfaces can be cleaned with most laboratory detergents. Dilute the cleaning agent as recommended by the manufacturer. Do not expose painted surfaces to concentrated acids or alcohols for prolonged periods of time or use any solutions containing hypochlorite, such as bleach, on any of the stainless steel surfaces as damage may occur.

- DO NOT use acetone to clean the plastic lenses (focusing lenses or upper lenses). Avoid any harsh treatment.
- During abnormal operation (Electrical or Mechanical Failure), Immediately contact your Maintenance department or our office.

Veriti Thermal Cycler

Thermocyclers, or thermal cyclers, are instruments used to amplify DNA and RNA samples by the polymerase chain reaction. The thermocycler raises and lowers the temperature of the samples in a holding block in discrete, pre-programmed steps, allowing for denaturation and reannealing of samples with various reagents. Amplified genetic material can be used in many downstream applications such as cloning, sequencing, expression analysis, and genotyping.

Standard operating procedure

1. Power On the Veriti Thermal Cycler from back side of the instrument.
2. Open the Lid.
3. Place samples (PCR tubes with reaction mixture) in the 96 well blocks.
4. Close the lid.
5. Select appropriate protocol to run.
6. Run the sample.
7. System will show the total time to finish the experiment.
8. After end of run, finish the run from screen.

9. Shut down the equipment from back side power button.
10. Open the lid
11. Remove the sample.
12. Close the Lid.

E-Gel® Imager Gel Documentation System

The Invitrogen™ E-Gel™ Imager is a compact, affordable, easy-to-use imaging system for stained gels and Invitrogen WesternDot™ 625 probe–stained western blots. Equipped with a high-resolution digital camera, the E-Gel system can be used to image gels incorporating fluorescent stains (e.g., ethidium bromide and Invitrogen™ SYBR™ stains) or colorimetric stains (Invitrogen™ Coomassie™ and silver stains). Additionally, you can image western blots detected with the WesternDot 625 detection kit reagents, avoiding the need for film processing. Setup is simple, and the system features intuitive software for band analysis.

Standard Operating Procedure

The system is provided with a Quick Reference Guide that includes the operating instructions.

General Guidelines: Wear proper safety equipment (ie. Lab coat, goggles, and protective gloves) when conducting experiments.

1. Connect the E-Gel® Imager system with power.
2. Launch the GelCapture software fromyour PC desktop.
3. Click Select to indicate the type ofE-Gel® Imager Light Base

being usedto provide transillumination, andwhether the E-Gel® iBase™ Device isbeing used with it, OR if the E-Gel®iBase™/E-Gel® Safe Imager™, or E-Gel®Go! is being used with the E-Gel®Imager Adaptor Base.

4. Verify the appropriate coloredemission filter being used in the filtertray.

5. Remove the E-Gel® Imager CameraHood from the E-Gel® Imager Base.

6. Position the gel in the center of theE-Gel® Imager Base.

7. Place the E-Gel®Imager CameraHood on top ofthe E-Gel® ImagerBase.

8. Turn the cameraon.

9. Turn the E-Gel® Imager Base on.

10. When the camera and transilluminator are turned on, the image of the gel is displayed on the Live Mode Screen in real time.

11. Verify that the type of filter in the filter tray is appropriate for the type of stain being used in your gel sample.

12. Verify that the manual focus and irisdials on the camera hood are adjusted to the recommended settings for the type of E-Gel® Imager Base being used, as shown on the computer screen.

13. Perform fine adjustment of the image to attain the brightest image possible by clicking on either the minus or plus buttons, or sliding the gauges for each setting.

14. If bands of both high and low intensity exist in the gel, perform Extended Detection.

15. Define the region of the gel that you wish to image using the Area of Interest icon.

16. A green rectangular frame pops uparound the image. Drag the edges ofthe green rectangle with your cursorto select your Area of Interest.

17. Right click in the green AOI frame todisplay a menu with the AOI options.

Note: The options in the menu differdepending on whether you arelooking at a real-time image, orworking on a previously saved image.

18. Click the Save button to capture the image of your gel in TIFF format.

19. To save an image in BMP or JPEGformat, click Export and select BMP orJPEG.

20. The Save window appears.

21. Browse to the location where you want the image to be saved, and entera name for the image.

22. Click the Save button to save the file tothe selected directory.

23. Additional functions that can beperformed at this point include:

- **Print:** Sends the image to a printer.
- **Analysis:** Sends the image to the analysis software (see Gel Quant Express manual). Make sure that the GelQuant Express Software Activation Dongle is inserted in your computer before using this function.
- **Edit Image:** Allows the user toperform advanced applications on the image.

Multiple Image Acquisition: To capture a series of images over aperiod of time

24. Switch off the E gel imager base power and Camera.

25. Shut down the PC.

Good Laboratory Practices

Good laboratory practice or GLP is a set of principles intended to assure the quality and integrity of non-clinical laboratory studies that are intended to support research or marketing permits for products regulated by government agencies. The term GLP is most commonly associated with the pharmaceutical industry and the required non-clinical animal testing that must be performed prior to approval of new drug products. Good Laboratory Practice (GLP) deals with the organization, process and conditions under which laboratory studies are planned, performed, monitored, recorded and reported. GLP practices are intended to promote the quality and validity of test data.Published GLP regulations and guidelines have a significant impact on the daily operation of an analytical laboratory. GLP is a regulation. It is not only good analytical practice. Good analytical practice is important, but it is not enough.

General Laboratory Rules

- All workers are responsible for maintaining their laboratory in a clean, tidy, safe condition.
- Always clean up after yourself, as a cleaner or work colleague may not know what the spill is.
- When you leave the Department, you are responsible for the safe disposal of all of your chemicals, solvents, cultures, etc.
- Food and drink must not be consumed in laboratories or be stored in laboratory refrigerators or freezers.
- Eating, drinking, smoking, handling contact lenses, or applying cosmetics is not permitted in the laboratories.
- Wash your hands after handling chemical materials, after removing gloves , and before leaving the laboratory.
- Mouth pipetting is forbidden. Mechanical pipettes are provided instead.

- All procedures are to be performed carefully to minimize the creation of splashes or aerosols.
- High risk work should only be performed during working hours when other members of staff are present. Working after hours should only be done if it is unavoidable and on SOP's for which risk assessments deem the risk to be low and manageable. The Supervisor is responsible for assessing the risk of work being carried out and whether the person undertaking the work is competent.
- If a piece of equipment breaks down or needs maintenance, make sure it is decontaminated before asking someone to work on it. Do not continue using a piece of equipment that seems faulty or try to repair it yourself; report it to the Laboratory Scientist.
- Hypodermic needles should never be re-capped or removed from syringes. Simply place them in a sharps bin as soon as you have finished with the procedure.
- Before using a piece of equipment for the first time, study the instruction manual and seek training by an experienced operator. If in doubt, speak with your Laboratory Scientist or PI.
- Fume cabinets must not be used as storage areas.
- Turn equipment off when not in use.
- After finishing an experiment, or when taking a break or going home, clean up as follows:
  - Replace tops on solutions and return containers to appropriate places.
  - Replace lids on all pipette canisters.
  - Rinse and decontaminate all dirty glassware and place in trolley for wash up.

- ❖ Turn off equipment.
- ❖ Wipe down benches, close windows and doors, and turn off lights.

Important

All lab workers who will be working in the laboratory must review this SOP and sign the associated training sheet. Lab workers must have specific training on the proper handling of chemicals and must understand the hazards. In addition, all workers should review the SOP for each specific hazard class and/or process with which they will beworking.

Lab workers must demonstrate competence to the Laboratory Scientist/Incharge or designee by being able to:

1) identify the hazards and list any particularly hazardous handling techniques (use of a Schlenk line, rotary evaporation, canula transfer, extremes of pressure or temperature, etc.),

2) list the foreseeable emergency situations,

3) describe the proper response to the emergency situations, and

4) know the appropriate control measures to minimize the risks.

When working in the lab, a laboratory worker must:

1) not work alone;

2) be cognizant of all of the SDS and safety information presented in this document;

3) follow all related SOPs in the laboratory SOP bank (PPE, syringe techniques, waste disposal, etc. as appropriately modified by any specific information in the SDS information presented in this document);

4) employ no more than the approved amounts of chemicals in any given reaction (larger quantities REQUIRE the approval of PI or designee), and

5) discuss ALL issues or concerns regarding chemicals with the PI prior to their use.

If there is an unusual or unexpected occurrence when using these materials or processes, the occurrence must be documented and discussed with the Principal Investigator or Lab Supervisor and others who might be using the same chemical or process. Unusual or unexpected occurrences might include a fire, explosion, sudden rise or drop in temperature, increased rate of gas evolution, color change, phase change, or separation into layers.

Prior Approval/review Required

All work with hazardous chemicals must be pre-approved by the Lab Scientist/Incharge and all training must be well documented. In addition, the following shall be completed:

- ❖ Document specific training on the techniques and processes to be used.
- ❖ Read and understand the relevant Safety Data Sheet.
- ❖ Demonstrate competence to perform the work.

A review of this SOP and re-approval is required when there are any changes to procedures, personnel, or equipment, or when an incident or near miss occurs.

Waste Disposal

All waste must be disposed of through the Hazardous Waste Program/Protocol.

Staff dealing with hazardous waste disposal should have completed Biomedical Waste management Training at KGMU Institute/Lab

General hazardous waste disposal guidelines:

- ❖ Affix hazardous waste on all waste containers as soon as the first drop of waste is added to the container.

- Store hazardous waste in closed containers, in secondary containment, and in a designated location. Do not let product enter drains. Discharge into the environment must be avoided.
- Double-bag dry waste using transparent bags.
- Waste must be under the control of the person generating and disposing of it.
- Dispose of routinely generated chemical waste within 90 days.

SOP FOR PCR

1 Set up the reaction wearing gloves at all times and label the lids of the eppendorf, as anything written on the sides will not remain after being in the PCR machine.

2 Take care to avoid carry over between tubes or contamination of stock

Note: Include the following control reactions for each DNA template.

(i) Positive DNA control if appropriate

(ii) Negative DNA control if appropriate

(iii) No template control using sterile water instead of DNA

3 A typical reaction is as follows:

Sterile pure water 42µl

10 x AJ buffer 5µl

Primer 1 (Forward) 10pm/µl 1µl

Primer 2 (Reverse) 10pm/µl 1µl

Template DNA or cDNA 1µl

50µl

Note: When using cDNA preparation containing Dynabeads as

template, ensure complete mixing of the bead suspension before removing an aliquot for PCR.

4. Spin the tubes briefly in a microfuge

5. Place the eppendorf in the thermal cycler and carry out an initial denaturation at 98°C for 3 minutes.

6. Cool the PCR machine to 80°C (or annealing temp) and pause it at this temperature

Add 1 unit of Taq polymerase (1 l of Taq diluted 1:5 or 2 l of a 1:10 dilution in 1 x AJ buffer)

7. Re-start the machine and amplify the DNA for between 25 - 40 cycles.

Parameters for a typical cycle might be:

94°C 30 sec. Denaturation

60°C 30 sec. Annealing of primers

72°C 30 sec. Extension of sequences

8. Remove tubes from Thermal cycler.

9. Analyse the PCR products on a 2% agarose gel, as described in SOP

Standard Operating Procedure For Agarose

Section - 1

All work with Ethidium Bromide (EtBr) is confined to the gel running and pouring benches and the gel doc areas (which are labeled). When you leave the Ethidium Bromide area you MUST remove your gloves to prevent EtBr being transferred to other parts of the lab.

- Do all of these steps using nitrile gloves!!
- Measure out agarose powder and add appropriate amount of1x TAE buffer
- Boil until all agarose has been dissolved and allow to cool until warm to touch.
- Add 4ul of EtBr stock solution directly to the get tray in the fume hood and then add 50ml of liquid agarose using filter tip. Mix the EtBr into the gel by rocking the tray gently.
- Dispose of filter tip in EtBr waste bucket located in the fume hood.
- Use Gel Doc system to visualize gel. Do not drip buffer when transporting the gel!
- Dispose of EtBr gel in drying tray in the fumehood located in.

Section -2 Hazardous Chemicals

Ethidium Bromide, (Et Br) is a mutagen and must be handled carefully, Read a MSDS from manufacturer and EH&S fact sheet on DNA stains:

http://www.ehs.berkeley.edu/pubs/factsheets/47ethidiumbromide.pdf

Section 3 – Potential Hazards

- Mutagen (may cause genetic damage) at high and low concentrations
- Toxic when working with stock solutions, powder or crystals.
- Avoid working with Ethidium Bromide in the raw form (powder or crystal). Order pre-diluted stock solutions.

- Irritant to the skin, eyes, mucous membranes, and upper respiratory tract when working with stock solutions and powders.

Section 4 – Approvals Required

Permission from Lab Incharge

Section 5 – Designated Area

Use EtBr in a designated area, this area is . When working in this area wear gloves and wash before eating.

- EtBr stock solution is located in room ___ in the chemical container. Two 1 mL aliquots are provided in the Ethidium Bromide working area (these workspace are labeled).
- Ethidium Bromide powder is NOT used in this lab we use premixed solutions

Section 6 – Special Handling Procedures and Storage Requirements

- Recommend wearing safety glasses and gloves when handling gels and buffer solutions.
- Short wave ultraviolet radiation will harm your eyes and skin therefore make sure the gel doc is closed before turning UV on (note there is safety system to help prevent this from happening but it is good practice not to do it anyway).
- EtBr stock solutions and powder should be stored away from strong oxidizing agents in a cool, dry place and the container must be kept undamaged and tightly closed.

Section 7 – Personal Protective Equipment

- Wear nitrile gloves, along with Lab coat and safety goggles which are provided in room _____.
- When dealing with Ethidium Bromide also use the fume hood when possible.

Section 8 – Engineering/Ventilation Controls

- DO not switch on/off any electrical items.
- Area should be well ventilated

Section 9 – Spill and Accident Procedures

1. Put on heavy duty gloves (preferred, since abrasive pad may puncture thin nitrile gloves) or double layers of nitrile gloves.
2. Soak the spill up with paper towels or some other absorbent and place absorbents in a secondary container that does not leak.
3. Once the area is dry, spray the affected area with scrub the area with a steel wool or brillo pad for several minutes.
4. Using paper towels dry up the area and then wipe the area down with absorbents dipped in tap water. Repeat this process until the area is clean.
5. Using a UV light, check the area to ensure that all the Ethidium Bromide has been removed. Repeat decontamination procedure as necessary.
6. Place all contaminated towels, pads and other debris in a secondary container (non-leaking) with a lid. Label as "Hazardous Waste, Ethidium Bromide (Mutagen)".

Note: Full protective equipment (as described in "Personal Protection" above) should be worn when cleaning up spills of stock solution, powder or crystals.

Emergency Exposure Procedures:

Note: all spills involving human skin, eye or mouth contact must be reported. The victim should immediately seek medical evaluation at the Medical Emergency, KGMU

Eye: If EtBr comes in contact with the eyes, immediately flush them with copious amounts of cold or cool water for a minimum of 15 minutes, preferably in an emergency eye wash.

Skin: In the event of skin exposure, remove contaminated clothing and immediately wash the affected area with copious amounts of cold or cool water for a minimum of 15 minutes.

If swallowed or inhaled: In the case of EtBr ingestion, obtain medical attention immediately. If EtBr is inhaled move the victim to a source of fresh air.

Section 10 – Waste Disposal

- Ethidium Bromide Stock Solutions and Powder- or Crystal-contaminated materials must be managed and disposed of as a hazardous waste.
- Agarose gels with trace amounts of Ethidium Bromide (0.3 - 0.5 µg/ml) are to be dried (in fume hood) and then packaged into bags for disposal.
- **Buffer Solutions** with trace amounts of Ethidium Bromide must be added to the large container in the fume hood which contains charcoal absorber, stir overnight and recheck for EtBr using hand held UV.

- **Pipette tips** can be disposed of in Ethidium Bromide waste bin and will be removed as per dried gels. NOTE: Pipette tip collection bin must be closed when not in use.

Section 11 - Decontamination

1. Put on heavy duty gloves (preferred, since abrasive pad may puncture thin nitrile gloves) or double layers of nitrile gloves.
2. Soak the spill up with paper towels or some other absorbent and place absorbents in a secondary container that does not leak.
3. Once the area is dry, spray the affected area with scrub the area with a steel wool or brillo pad for several minutes.
4. Using paper towels dry up the area and then wipe the area down with absorbents dipped in tap water. Repeat this process until the area is clean.
5. Using a UV light, check the area to ensure that all the Ethidium Bromide has been removed. Repeat decontamination procedure as necessary.
6. Place all contaminated towels, pads and other debris in a secondary container (non-leaking) with a lid. Label as "Hazardous Waste, Ethidium Bromide (Mutagen)"

Summary

- If I have any questions I must ask a trained person before working with Ethidium Bromide and making gels.

- I understand that Ethidium Bromide is a hazardous substance and that I must work in a manner that puts my own safety first.
- I understand that personal safety equipment must be worn at all times and that all work must be done in the fumehood – no exceptions.
- I must also ensure the safety of my colleagues – bottles of EtBr and EtBr contaminated material will not be placed or used in a way that might make contact with other lab personnel.

I have read and understood the above.

Name (Printed)	Signature	Date

Special Handling Procedures And Storage Requirements

1. Wash thoroughly after handling any contaminated material, chemical, or waste.
2. All chemical containers must have a legible, firmly attached label showing the contents of the container. Labels on incoming containers of hazardous chemicals must not be removed or defaced. Any labels that are damaged must be immediately replaced with labels containing the same identification, warnings, and source information.
3. A hazard review of new materials not previously used in the laboratory must be completed under the direction of the scientist/incharge before actual handling of the material begins.
4. Chemical substances (or by-products) developed in the laboratory are assumed to be hazardous in the absence of other information.
5. Store all chemicals in a tightly closed, labeled container, and in a cool, dry, well ventilated area. Segregate from incompatible materials. Secondary containers must be labeled clearly. Follow any substance-specific storage guidance provided in Safety Data Sheet documentation.
6. Use small quantities whenever possible. Monitor your inventory closely to assure that you have tight control over your material

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