UMassAmherst | Environmental Health & Safety

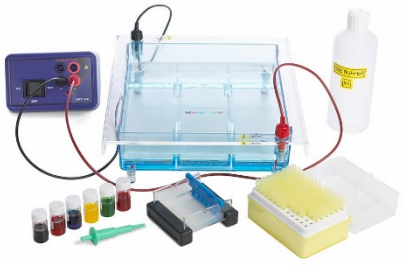
Acrylamide and Agarose Gels and Stains SOP

Summary

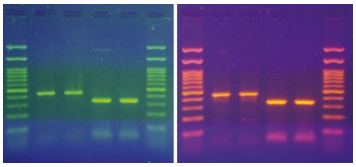
- Acrylamide, ethidium bromide, and other stains used in gels are hazardous materials that can cause long term health effects.
- Casting gels can present hazards from chemical exposure and burns when heating.
- Running gels presents hazards of electrical shock.
- Gels must be disposed of properly and not in the normal trash.

What are acrylamide and agarose gels?

Gel electrophoresis is a technique used to separate mixtures of DNA, RNA, and proteins based on molecular weight and charge. By providing an electrical charge across a stationary phase, charged samples are separated as they move through a gel medium, which influences sample migration based on size and charge of the analytes and porosity of the medium. Gels are typically made of agarose or polyacrylamide of varying percentages dissolved in a buffer and solidified or polymerized, respectively. Stains are incorporated into the gel to allow for sample visualization.



Acrylamide is most commonly used in the production of polyacrylamide polymers. Apart from its wide use in the manufacturing industry, it is commonly used in laboratories for the purpose of polyacrylamide gel electrophoresis (PAGE) that is typically used to separate proteins and peptides based on electrophoretic mobility. A common stain used to detect proteins is Coomassie blue, which typically consists of a solution of Coomassie blue dye in glacial acetic acid and methanol. The dye turns from red unbound in solution to blue when it associates with proteins through non-covalent interactions. Dyes containing silver and various fluorescent tags are also often used.



Agarose is made up of polysaccharides and is commonly used in laboratories for the purpose of agarose gel electrophoresis for the separation of DNA and other polynucleotides. Aqueous solutions of ethidium bromide are commonly used in agarose gel electrophoresis for the purpose of visualizing nucleic acid bands. Ethidium bromide fluoresces orange upon exposure to UV light. It is a flat, planar, and aromatic compound capable of intercalating, or inserting between DNA base pairs (which are also flat, planar, and aromatic), and the resulting complex structure is stabilized by π stacking. Fluorescent intensity of ethidium bromide increases dramatically once intercalated versus the

emission from solution phase solvated form thus enabling visualization. Though ethidium bromide is the most popular type of fluorescent intercalating stain, others such as SYBR safe are widely used as well.

What are the hazards?

Ethidium bromide and acrylamide are both hazardous chemicals as defined by the OSHA Hazard Communication Standard described in 29 CFR 1910.1200(d), and are the primary chemical safety concern when creating or working with gels in labs. Exposure can occur via inhalation (if aerosolized), ingestion, and skin absorption. Because of its ability to bind to DNA, ethidium bromide is a potent mutagen capable of causing genetic damage. Acute exposure can cause irritation to the eyes, mouth, skin, and upper respiratory tract.

The monomer acrylamide is a probable human carcinogen and may also cause adverse reproductive and nervous system health effects. While toxicity and potential for exposure substantially decrease after polymerization, exposure remains a concern as complete polymerization cannot be assured. Exposure to acrylamide can cause both chronic and acute health effects including ataxia (loss of muscle coordination), numb limbs, weakness, and drowsiness.

Physical hazards are presented during the heating portion of agarose gel preparation. Spilling and splashing may occur during the heating, mixing, and pouring of the hot liquefied gel, which poses a burning hazard to the body including the face, hands, and upper body. Electrocution is also a potential hazard as typical voltages of 100 V can be applied across gels resulting in 25 mA of current.

Occupational Exposure Limits

The Permissible Exposure Limits (PELs) for acrylamide according to OSHA and ACGIH are 0.3 mg/m^3 and 0.03 mg/m^3, respectively. Workers should not be exposed to values exceeding the PEL over the course of an 8-hour work day. PELs for ethidium bromide have not been established. For more information, please refer to OSHA PELs Table Z-1 (https://www.osha.gov/dsg/annotated-pels/tablez- 1.html), NIOSH Pocket Guide to Chemical Hazards (https://www.cdc.gov/niosh/npg/default.html) and ACGIH® 2019 Threshold Limit Values.

What Activities Could Pose a Risk?

Laboratory workers are at risk for exposure when making gels using either ethidium bromide or acrylamide. Inhalation exposure is a concern especially when working with the pure, solid forms of these chemicals, as dust can easily be dispersed throughout the air during operations such as pouring, weighing, mixing, or any such manipulations. Staining and destaining operations can also lead to solvent exposures if these are not conducted with appropriate containment (i.e., in a fume hood).

The process of heating and mixing the elements required for agarose gel electrophoresis poses a risk for splashing and burns to the body as well as dermal exposure to ethidium bromide, as it is typically pipetted into the mixture directly after heating. The flask containing the mixture is very hot and may lead to burning of the hands if proper protection is not used. Swirling the heated mixture and moving the flask close to the face when checking for complete dissolution of the agarose powder may lead to

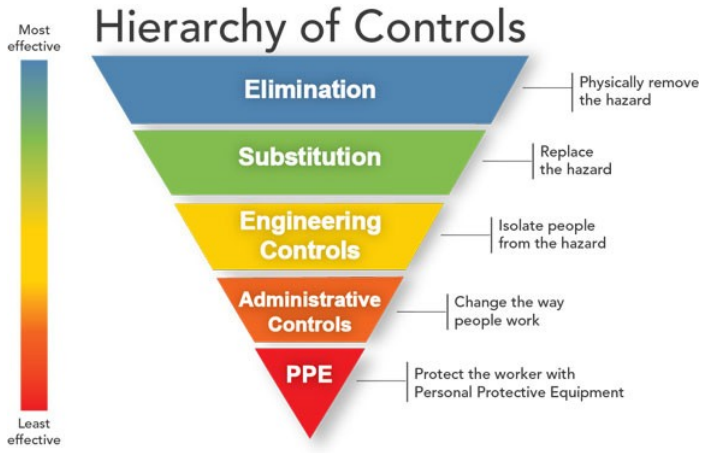
splashing of the mixture into the face, hands, arms, and upper body leading to burns and dermal exposure to ethidium bromide. Pouring the hot mixture into the gel casting tray may also lead to splashing onto hands and arms.

The process of heating the mixture in a microwave and relying on the occurrence of boiling as an indicator of completion can also pose a hazard as the mixture may not appear to boil even if it is very hot and the powder fully dissolved. Once the powder is fully dissolved during the heating process, no material remains to serve as a source of nucleation for boiling, which can enable the mixture to superheat and flash boil, especially if it is moved directly after heating. If no boiling is observed, researchers might continue to heat the mixture unintentionally causing it to superheat, which can lead to flash boiling and splattering.

Running gels can also pose a potential for electrocution if the equipment is not in good working order, connected improperly, mishandled, or improperly grounded.

How Can Exposures Be Minimized?

As with any other hazardous material, always conduct a thorough risk assessment and employ the hierarchy of controls to minimize risk when working with acrylamide, ethidium bromide, or when heating agarose mixtures in the microwave. Some specific applications of the hierarchy of controls to these hazards are listed below.



Always apply the controls in the order of most effective to least effective (see graphic), and apply as many controls as possible to reduce the risk to the lowest achievable level.

Elimination/Substitution

- Replace high concentration products with low concentrations or less hazardous substitutes, whenever possible.
- Purchase premade solutions of ethidium bromide and acrylamide at desired concentrations to avoid working with the solid forms which are more concentrated and pose a greater inhalation hazard.

Engineering Controls

- Avoid inhalation by using engineering controls where possible in dispensing, mixing, and pouring areas, such as local exhaust ventilation (e.g. chemical fume hood). Work with solid ethidium bromide and acrylamide should always be conducted in areas with adequate ventilation or containment. Local enclosures and fume hoods are good options. All work with solvents for staining and destaining should be conducted in a fume hood.
- Routinely maintain local exhaust ventilation systems and check that chemical fume hoods are operating at the proper face velocity before each use.
- Ensure that laboratories are negatively pressurized with respect to the hallway.

Administrative Controls

- Before use:
  - Consult the manufacturer's Safety Data Sheet and additional chemical information at https://cems.unh.edu/umass/CEMS/SearchSDS.
  - Locate nearest eyewash and shower and confirm that they are accessible and within 50 ft. of the work area. This is particularly important when working with corrosive stains (e.g., coomassie).
  - Ensure all gel apparatus is in good working order. Do not use damaged connectors or cables that are frayed. Use power supplies that have load protections. Follow the manufacturer's directions regarding assembly and use of gel apparatus.
- If weighing solids of ethidium bromide or acrylamide, take an aliquot from the bulk supply that closely approximates the amount needed and place in a closed container while working in a fume hood or local enclosure. It is best to tare the container prior to use and then make final weight adjustments very carefully at the balance to avoid dust formation.
- Designate and label areas in the lab where ethidium bromide is used and stored.
- Cover areas where gels are to be poured with a bench cover and change out when it becomes contaminated or is spilled on.
- Clean areas where hazardous materials are weighed, dispensed, or used on a regular basis to reduce accidental exposure.

- When heating liquids in the microwave, use boiling stones to ensure that a nucleation site is always present in heated solutions that do not have convective forces capable of initiating nucleation (i.e., constant stirring or shaking). Teflon boiling stones are chemically resistant and do not contaminate solutions: https://www.fishersci.com/shop/products/saint-gobain-chemware-ptfe-boiling-stones/0919120#?keyword=teflon+boiling+stones. Adding 4-5 stones to each batch of agarose will prevent superheating and flash boiling.
- Always wait a few moments after heating liquids in a microwave before moving to lower the probability that any localized super heating will result in flash boiling.

Personal Protective Equipment

- Always wear appropriate PPE when making gels including:
  - Safety glasses or chemical splash goggles and face shield (when heating liquids in a microwave)
  - Lab coat and closed toed shoes
  - Nitrile gloves
  - Thermal gloves when handling hot materials
- In cases where engineering controls do not provide sufficient protection for inhalation exposure, please contact EH&S. We will work with you to determine the appropriate respirator based on a risk assessment for work conducted using acrylamide, ethidium bromide, or other hazardous materials. Respirator users must enroll in the University's Respiratory Protection Program

(https://ehs.umass.edu/respiratory- protection-program).

Waste Handling

Treat waste as hazardous and dispose of appropriately. Each of the following waste streams should be kept separate and referred for disposal through CEMS https://cems.unh.edu/umass/CEMS/RequestRemoval:

- Contaminated debris (e.g. bench covers, pipette tips, gloves) and used gels should be bagged and placed in a labelled solid hazardous waste container. Contaminated glass should be placed in a puncture resistant container before placing in the solid waste hazardous container.
- Solid forms of ethidium bromide and acrylamide can be disposed of as hazardous waste in their original container or in a sealed, labelled bag.
- Stock solutions of ethidium bromide and acrylamide can be disposed of as hazardous waste in their original container.
- Buffers containing any amount of acrylamide or ethidium bromide should be referred for hazardous waste disposal through CEMS.

Exposure and Spill Procedure

In the event of a spill involving ethidium bromide, acrylamide, or other hazardous material that does not involve the contamination of a person, the material may be cleaned up if it is safe to do so following the general procedure for small spills detailed in the University's Chemical Hygiene Plan.

- Ensure that it is safe to clean up the spill.
    - Spills of more than 1 L should be immediately referred to EH&S (413-545-2682) and the area should be evacuated. Do not permit entry to the area until EH&S arrives.
- Ensure that cleaning up the material will not generate airborne dust or aerosols.
    - Spills of highly volatile solvents or hazardous powders outside of enclosures (fume hoods or other enclosure devices) should be immediately referred to EH&S (413-54-2682) and the area should be evacuated. Do not permit entry to the area until EH&S arrives.
- Use absorbent material to contain and remove the liquid. After the liquid is removed rinse the area with water and contain and remove with absorbent material.
- Place all items used for cleanup in a labeled hazardous waste container and request a pickup through CEMS.
- If at any point you are uncomfortable cleaning up the spill or require assistance, stop and call EH&S (413-545-2682).

Exposures to hazardous materials should follow the general procedures for exposures outlined in the University's Chemical Hygiene Plan.

For a major exposure requiring the use of a drench shower or eyewash:

- Have someone call 911 (report the building name, room number, and street address) or 413- 545-3111 (or simply 5-3111 from a campus line) to report the incident and request

medical help. If possible, communicate to the first responders the nature of the exposure (e.g. ethidium bromide, acrylamide, solvents, corrosives, hot materials).

- Help the affected individual to position their head over the eyewash and activate it, or position them under the drench shower and activate it as appropriate.
    - Always ensure your own safety before helping others. Only help if it is safe for you to do so.
    - Wear gloves, safety glasses, and a lab coat.
- If using an eyewash: Instruct the affected individual to open their eyes and roll them around while the water is flowing. Help them to hold their eyes open if necessary and safe to do so.
- If using a drench shower: Remove all clothing from the affected area while under the shower.
- Flush the affected area for 15 minutes with water.

For minor exposures such as a spill to readily accessible extremities (e.g., hand):

- Flush the affected area in a sink equipped with potable water for at least 15 minutes.
- Go to University Health Services (UHS) for medical evaluation, and tell them you have had a lab exposure.
- Notify EH&S (413-545-2682) as soon as possible and complete the lab incident form (https://ehs.umass.edu/lab-incidents-and-lab-incident-report-form).

References and Additional Resources

1. National Institute of Environmental Health Science. https://www.niehs.nih.gov/health/topics/agents/acrylamide/index.cfm
2. The National Institute for Occupational Safety and Health. https://www.cdc.gov/niosh/topics/acrylamide/
3. Millipore Sigma Ethidium Bromide Safety Data Sheet. https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=E1510&brand=SIGMA&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2FSIGMA%2FE1510%3Flang%3Den
4. Agency for Toxic Substances & Disease Registry. Toxic Substances Portal – Acrylamide. https://www.atsdr.cdc.gov/phs/phs.asp?id=1113&tid=236