EHS Laboratory Guidance

Ethidium Bromide and Alternative DNA Stains: Precautions and Waste Disposal Procedure

Ethidium bromide (EtBr) is commonly used as a non-radioactive DNA stain to identify and visualize nucleic acid bands in electrophoresis and perform other methods of nucleic acid separation. EtBr is a dark red, crystalline, non-volatile powder that is moderately soluble in water. Solutions of EtBr fluoresce readily with a reddish-brown (605 nm) color when exposed to ultraviolet (UV) light (absorption maxima at 210 nm and 285 nm). Although it is an effective tool for genomic research, its hazardous properties require special safe handling and disposal.

There are a number of alternative DNA stains available, including SybrSafe, EZ Vision and Gel Red. DNA stain alternatives may be better for DNA visualization and less hazardous than EtBr; however, anything capable of binding DNA with high affinity is a possible mutagen. Only a few alternatives to EtBr have been thoroughly tested, so toxicological data is lacking. Environmental Health & Safety (EHS) has reviewed the available data, consulted with DNA stain manufacturers and other universities, and summarized our findings in this guidance document.

EtBr is a potent mutagen (may cause genetic damage), and moderately toxic after an acute exposure. EtBr can be absorbed through skin, so it is important to avoid any direct contact with the chemical. EtBr is an irritant to the skin, eyes, mouth, and upper respiratory tract. It should be stored away from strong oxidizing agents in a cool, dry place, and the container must be kept undamaged and tightly closed because EtBr may sublimate.

Some alternative stains have been found to be less mutagenic and less toxic than EtBr. If the toxicological data is lacking or unclear, the stain should be handled in the same way as EtBr. Some alternative stains are suspended in dimethyl sulfoxide (DMSO), which has health implications of its own, including increased skin absorption of organic compounds.

Good laboratory work practices help reduce hazardous exposures.

- To prevent inhalation exposure, work with powder or crystals in a fume hood, or use **premixed** solutions or tablets.
- To prevent skin contact, wear nitrile gloves, a laboratory coat, long pants, and closed-toed shoes. Change gloves frequently.
- Provide users with safety training on the hazards, use, and proper cleanup procedures. Document training by having users sign the unit's Laboratory Safety Plan (LSP). Refer to this fact sheet in the LSP as a standard operating procedure.
- Review the Safety Data Sheet (SDS) and this EHS fact sheet before handling DNA stains.

- Wear eye protection and ensure that there is unobstructed access to an emergency eyewash/shower unit in the work area.
- As with any chemical, to avoid ingestion do not eat or drink where DNA stains are handled, processed, or stored.
- Always wash hands after handling, even if gloves were worn.
- Wear UV-blocking eyewear or work behind a UV shielding glass when using ultraviolet light.
- Careful housekeeping is necessary when working with DNA stains. Delineate and restrict the area in which DNA stains may be used. Check other areas with UV light in a darkened room and follow the decontamination procedures below for contaminated surfaces.

Emergency Procedures:

Eye care: If EtBr comes in contact with the eyes, immediately flush them with copious amounts of cool water for at least 15 minutes, preferably in an emergency eyewash.

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

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

Note: After any exposure to EtBr (via skin, inhalation, ingestion, or eye contact), the victim should immediately seek a medical evaluation from the Gritman Medical Center or Student Health Services/Moscow Family Medicine. Bring a Safety Data Sheet (SDS) to the clinic.

Spill Cleanup and Decontamination Procedures:

Unauthorized releases of DNA stain to sinks or laboratory room floor drains must be immediately reported to EHS by calling (208) 885-6524 during normal business hours or Security Services at (208) 885-7054 after hours. In the event of a large spill, notify all others in the spill area to stay away. Evacuate the immediate area and post signs warning others of the spill. Call 911 if there are any injuries or if the spill is too large to safely manage.

Small spills that do not enter drains can be cleaned up by laboratory personnel who are aware of the hazards, have been trained on the proper cleanup procedures, and have access to appropriate safety and cleanup equipment. If you do not have appropriate training or equipment, contact EHS for assistance.

- Always wear protective clothing when cleaning up a small spill.
- If the spill is **powder**, carefully collect the dry powder (because when wet it will stain) and then quickly wipe up residue with wet paper towels.
- If the spill is **liquid**, absorb freestanding liquid with dry paper towels.

- Use UV light in a darkened room to locate any remaining solution or stains, then clean area with strong detergent (e.g., tri-sodium phosphate). Use UV light and repeat decontamination as necessary.
- Contain and label the cleanup materials (e.g., "Laboratory debris contaminated with Ethidium bromide"), and follow the disposal guidelines on the following table.

Disposal:

The following table indicates which DNA stains are managed as if hazardous waste and how to dispose of them properly. Filtering methods are described below the table.
NOTE 1: Do not use "BIOHAZARD" bags to collect waste unless the material contains biological organisms or recombinant DNA that are not deactivated.
NOTE 2: The following disposal methods assume the waste does not contain a constituent that would make it a regulated hazardous waste.

Type of Unwanted Material	Disposal Procedures
Concentrated, unused or expired EtBr and other stains.	Though not a "regulated" hazardous waste in Idaho, these materials ARE managed as if hazardous waste. Dispose through EHS.
Solutions with EtBr, any concentration.	Though not a "regulated" hazardous waste in Idaho, these solutions ARE managed as if hazardous waste. Dispose through EHS.
SybrSafe, EZ Vision, EZ Vision Two, EZ Vision Three, GelRed and GelGreen: Non-toxic working solutions.	These dilute solutions are NOT considered hazardous waste. As a Best Practice, filter the solutions to prevent potential mutagens from entering our sewer system. See below.
Gels contaminated with EtBr ("Gel Waste").	Allow gels to dry out, and then place gels in lined plastic pail provided by EHS. Collect separately from other solid waste. Dispose through EHS.
Gloves, absorbent paper, cleanup debris contaminated with EtBr ("Solid Waste").	Place in lined plastic pail provided by EHS. Place sharps in heavy-walled plastic container, then deposit in pail. Collect separately from gel waste. Dispose through EHS.
Gloves, absorbent paper, and clean up debris that is contaminated with SybrSafe, EZ Vision, GelRed and GelGreen.	Allow gels to dry out, and then place gels and debris in trash bags. Tie the bags and place them in the nearest dumpster.
Used activated charcoal filters. Note: the filters are potentially a flammable solid waste.	Collect separately from other waste. Place in a plastic bag (e.g. Ziploc bag) and dispose through EHS.
All other stain solutions, gels, and contaminated debris.	You may send EHS the SDS for guidance. If toxicological data is inadequate, the material must be disposed of in the same manner as EtBr.

Filter Solutions for Drain Disposal of non-EtBr stains:

Prior to drain disposal of non-EtBr stains (SybrSafe, EZ Vision, EZ Vision Two, EZ Vision Three, GelRed and GelGreen), EHS recommends filtering all dilute DNA stain solutions. The procedures may be incorporated into your laboratory SOP and training.

There are simple kits available for charcoal filtration. Examples include:

1. Schleicher and Schuell supply a commercial filter funnel kit that uses a packaged charcoal disk that is graduated for easily tracking the amount of aqueous solution calculated for a fixed quantities of DNA stain (non-EtBr) residue. This is particularly useful for labs that generate large amounts of solutions at a time. The kit is available through VWR and other suppliers.

    - Filter the DNA stain solution (non-EtBr) through the charcoal filter.
    - Pour filtrate down the drain.
    - Place charcoal filter in a sealed bag (e.g., Ziploc) and dispose through EHS.
      Note: the activated charcoal may be considered a flammable solid.

2. Another simple charcoal filtration method is the Green Bag. The Green Bag® Kit allows rapid and trouble-free concentration of DNA stain solutions from large volumes of solutions into a small "tea" bag containing activated carbon which is then conveniently disposed along with other solid hazardous wastes. One kit has the capacity to remove 500 mg of DNA-stain from solutions (10 mg stain/bag).

    - Place the Green Bag into the DNA stain solution (non-EtBr).
    - Allow to sit for the allotted time.
    - Pour filtrate down the drain.
    - Place the used Green Bag(s) in a Ziploc bag.
    - Dispose of the Green Bag through EHS.

3. Amresco Destaining Bags are similar to the Green Bag Kits and remove up to 5 mg of DNA stains and other biological stains, including Coomassie Blue. Follow the same procedures as for the Green Bag kits.

After filtering, check the solution for fluorescence using UV light. If fluorescence is detected, treat the solution again until the fluorescence is not detectable. Do not exceed the extractor's capacity, as described in the product instructions. The filters resulting from filtration of non-EtBr solutions must be disposed through EHS. Label as "Filters containing SybrSafe" or other DNA stains as appropriate.

Call EHS at (208) 885-6524 for any questions.