

Environment, Health and Safety Information for the Berkeley Campus

## Ethidium Bromide and Alternative DNA Stains: Precautions, Waste Treatment and Disposal

### Ethidium Bromide

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 fluoresces readily with a reddish-brown color when exposed to ultraviolet (UV) light. Although it is an effective tool for genomic research, its hazardous properties require special safe handling and disposal.

### Ethidium Bromide Alternative

There are a number of alternative DNA stains available, including Sybr Safe, 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. An EH&S work group reviewed the available data, consulted with DNA stain manufacturers and other universities and summarized our findings in this fact sheet.

### Health Hazards and Safety Precautions

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.

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.

### Recommended Laboratory Work Practices

Good laboratory work practices help reduce hazardous exposures.

- To prevent inhalation exposure, work with powder or crystals in a fume hood, or work with **premixed** solutions or tablets to avoid inhalation exposure.
- 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 signing the laboratory's Chemical Hygiene Plan (CHP). Refer to this fact sheet in the CHP as a standard operating procedure.
- Review the Material Safety Data Sheet (MSDS) and this EH&S 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.



## Emergency exposure procedures

- 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 are worn.
- Wear UV-blocking eyewear or work behind a UV shielding glass when using ultra-violet 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.

**Eye care:** If EtBr comes in contact with the eyes, immediately flush them with copious amounts of cold or 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 cold or 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 Tang Center or, if the exposure occurs while Tang Center is closed, from Alta Bates Medical Center (2450 Ashby Avenue, Berkeley).

Unauthorized releases of DNA stain to sinks or laboratory room floor drains must be immediately reported to EH&S by calling 642-3073 during normal business hours or 642-6760 (UCPD) 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.

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 EH&S (or UCPD after hours) 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.

## Spill Cleanup and Decontamination Procedures



## Disposal

The following table indicates which DNA stains are considered hazardous waste and how to dispose of them properly. Filtering methods are described below the table.

Type of Unwanted Material	Disposal Procedures
Concentrated, unused or expired EtBr and stains .	Label and dispose of through EH&S.
Solutions with EtBr <0.15%	These dilute solutions are NOT considered hazardous waste. As a Best Practice, filter the solutions to prevent mutagens from entering our sewer system and bay. Bench Top Treatment requirements do not apply since this is not hazardous waste treatment.
Solutions with EtBr >0.15%	These solutions ARE considered hazardous waste. <a href="#">Dispose of them through EH&amp;S</a> OR filter them and follow <a href="#">Bench Top Treatment</a> requirements.
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 mutagens from entering our sewer system and bay. Bench Top Treatment requirements do not apply since this is not hazardous waste treatment.
Gels, clean up debris and used filters, contaminated with EtBr	Allow gels to dry out, and then place gels and debris in clear, labeled bags. <a href="#">Dispose through EH&amp;S</a> . (See the EH&S fact sheet on <a href="#">Sharps</a> if you have broken glass or sharps.)
Gels, clean up debris and used filters, 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 garbage. (See the EH&S fact sheet on <a href="#">Sharps</a> if you have broken glass or sharps.)
All other stain solutions, gels, filters and contaminated debris	You may send EH&S the MSDS for guidance. If toxicological data is inadequate, the material must be disposed of in the same manner as EtBr.

## Filter Solutions for Drain Disposal

Prior to drain disposal, EH&S recommends filtering all dilute DNA stain solutions. Filtering ethidium bromide solutions with concentrations of >0.15% is considered hazardous waste treatment and only allowed if [Bench Top treatment rules](#) are followed. The following peer-reviewed article is a good source of information: Analytical Biochemistry #162, [Ethidium Bromide: Destruction and Decontamination of Solutions](#), Lunn and Sanstone, (453-458), 1987. The procedures (or those from another peer-reviewed article) may be incorporated into your laboratory SOP and training:



## Funnel Kit

There are simple kits available for charcoal filtration, which is one of the treatment methods described in the articles above. Examples include:

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 ethidium bromide 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 ethidium bromide solution through the charcoal filter.
- Pour filtrate down the drain.
- Place charcoal filter in a sealed bag (e.g., zip-lock) and dispose of it through the Hazardous Waste Program.

## The Green Bag

([http://www.qbiogene.com/products/lab-tools/EtBr\\_page1.shtml](http://www.qbiogene.com/products/lab-tools/EtBr_page1.shtml))

Another simple charcoal filtration method is the Green Bag. The Green Bag® Kit allows rapid and trouble-free concentration of ethidium bromide 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 ethidium bromide from solutions (10mg EtBr/bag).

- Place the Green Bag into the ethidium bromide solution.
- Allow to sit for the allotted time.
- Pour filtrate down the drain.
- Dispose of the Green bag in the biohazardous waste box for incineration.

## Destaining Bags

[Amresco Destaining Bags](#) are similar to the Green Bag Kits and remove up to 5 mg of ethidium bromide 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.

Solids resulting from filtering EtBr solutions must be disposed through EH&S. Label as "solids containing ethidium bromide" (or other DNA stain as appropriate).

For complete instructions regarding proper disposal of items through EH&S, please refer to the EH&S Fact Sheet entitled "[Hazardous Waste Management](#)."

Call EH&S at 642-3073 for any questions.

