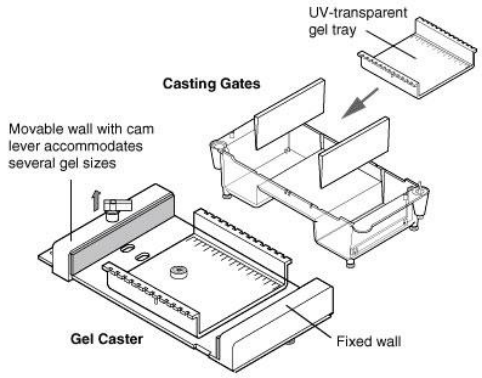
Ethidium Bromide (EtBr)

Ethidium Bromide (EtBr, CAS# 1234-45-8) is widely used in biochemical research laboratories for visualizing DNA fragments, and is primarily used on campus for agarose gel electrophoresis procedures. The dye intercalates between the stacked bases of nucleic acids and fluoresces red-orange (560 nm) when illuminated with UV light (260 to 360 nm). This allows very small quantities of DNA to be detected.

Three steps **(Fig. 1 & 2)** are involved in the electrophoresis process: (1) a gel is prepared with an agarose concentration appropriate for the size of DNA fragments to be separated; (2) the DNA samples are loaded into the sample wells and the gel is run at a voltage and for a time period that will achieve optimal separation; and (3) the gel is stained or, if EtBr has been incorporated into the gel and electrophoresis buffer, visualized directly upon illumination with UV light. **(EtBr is frequently added to the gel and running buffer prior to electrophoresis.)**



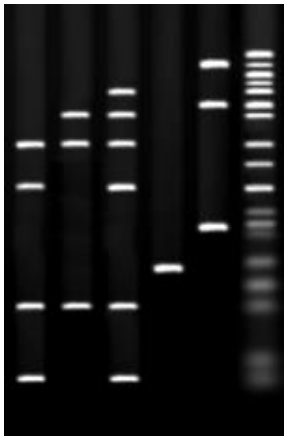


Fig.1: Minigel apparatus (*Reproduced with permission from reference* 2) **Fig. 2**: Bands in gels stained with ETBR fluoresce under uv-light

Health Hazards

Hazard Statements
Toxic if Inhaled. Suspected of causing genetic defects

EtBr is strongly mutagenic. Although there is not enough evidence yet that EtBr causes cancer or birth defects in humans, this material must be considered a possible carcinogen and reproductive toxin based on numerous cell and animal studies. EtBr is readily absorbed through

the skin and is highly toxic by inhalation, particularly in powder form, as well as irritating to the skin, eyes, mucous membranes and upper respiratory tract. EtBr may also cause extreme eye and skin irritation. There is no conclusive as to whether or not this chemical would be likely to cause an allergic skin reaction. If ingested it may cause gastrointestinal irritation with nausea, vomiting and diarrhea.

Important Note: *Due to possibility that EtBr may disturb the development of an embryo pregnant researchers should avoid working with EtBr powders and stock solutions. Additional precautions may be necessary for working with gels. Contact EH&S if you would like a review of procedures.*

Safe Handling

- **Don't use powdered EtBr**. This will generate dust that can spread and contaminate other surfaces. While tablets of EtBr are also available and are a better alternative to powders, purchasing solutions of EtBr is preferred to avoid the risk associated with solid manipulations such as weighing, solution prep, etc.
- **Don't microwave EtBr and EtBr containing gels** to avoid generating EtBr vapors.
- **Use a fume hood. S**tock solutions and any operations capable of generating EtBr dust or aerosols should be conducted in a fume hood to prevent inhalation.
- **Wear UV-blocking eyewear** or work behind a UV shielding glass when using ultra-violet light.
- **Use dedicated equipment.** A dedicated tray and space should be utilized in the lab for aliquoting the concentrated stock. The only objects that should come in contact with EtBr are: the flask; the gel tray; the gel combs; and the gel tank **(Fig.1 & 2)**.

Personal Protective Equipment

- **Always use chemical splash goggles** when handling concentrated EtBr or if there is any possibility of splash.
- **Protect your entire skin.** Long sleeve shirts, pants, and closed-toe shoes should be worn when working with EtBr
- A laboratory coat must also be worn.
- **Wear gloves at all times when handling powders, liquids, or gels.** Following glove removal, always rinse hands.
- Glove selection is very important. Latex gloves offer little protection from EtBr – nitrile gloves are much more effective. Viton, nitrile, or butyl gloves are also typically worn. **Safety *Tip:*** *While working with high concentrations or for a prolonged period of time, double-gloving your nitrile gloves can further reduce the risk of exposure, especially if the outer glove is replaced whenever significantly contaminated.*

Exposures to EtBr

- **Skin Exposure:** Immediately wash all the affected areas of skin. Using the sink may be appropriate for exposures to the hands and forearms, but contamination of the head, legs or torso should be handled with a safety shower. When using a safety shower remove all affected clothing.
- **Eye Exposure:** Immediately irrigate eyes at eyewash for at least **15 minutes** with copious quantities of water keeping eyelids apart. Seek prompt medical attention.
- **Inhalation:** Move the exposed person to fresh air. In all cases of overexposure through inhalation seek prompt medical attention.
- **Ingestion (Swallowing):** If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Seek medical aid.

Spill Clean-up and Decontamination

Small spills of EtBr solutions can be cleaned by lab staff. Individuals cleaning spills must wear appropriate PPE as described in the Personal Protective Equipment section of this document.

- Spills of EtBr solution should be absorbed into a neutral absorbent material (e.g. spill absorbent pads or paper towels) and the area should be decontaminated using the decontaminating chemical combination described below. Avoid formation of dust when cleaning up solid spills by slowly adding water and then absorbing the solution. All spill clean-up materials and absorbents should be double-bagged in polyethylene bags or placed in a sealed container and disposed of using EH&S pick-up request.

- For minor spills of low concentration (e.g. up to 10 mL of 10 mg/mL EtBr) after wiping up the spill, wipe the area down with a 50:50 mixture of ethanol and water. Any remaining surface streaks could be removed with a small amount of 2% bleach, if appropriate and compatible, followed by rinsing with water. **However, bleach must not be used directly on solid or liquid spills as this produces a highly toxic product.**

- The following method can also be used to decontaminate equipment and areas and could be of particular use after a severe spillage or for a challenging decontamination. Prepare the decontamination solution just prior to use.

  Add 20 mL of hypophosphorus acid (50%) (H_3PO_2) and 4.2 g. of sodium nitrite ($NaNO_2$) to 300 mL water. ***IMPORTANT:*** This must be done in a fume hood since a small amount of nitrogen dioxide may be released when the solution is initially mixed. Care should be taken due to the acidity of the solution (pH 1.8).

- Use proper PPE and prepare immediately before using.
- Wash the area/equipment at least four times with paper towels soaked in tap water, using a fresh towel each time.
- Soak all towels in the decontamination solution for 1 hour.
- Neutralize used decontamination solution and towels with sodium bicarbonate.
- Put used towels in a bag and place in a secondary bag or box prior to disposal.
- The used, neutralized solution can be poured down the drain with ample amount of water.

Safety Tip: *If needed, use a hand-held UV lamp to check for residual EtBr contamination subsequent to clean-up. A reddish-orange fluorescence can be detected under both 'long' and 'short' UV wavelengths. Remember that there are other hazards associated with the use of UV; all personnel must wear appropriate PPE to cover the skin and eyes.*

Training

Before working with EtBr, researchers should be trained in: properties of EtBr; general handling and storage techniques as well as the specific procedures involving EtBr; the appropriate personal protective equipment requirements; first aid procedures and emergency response and spill control procedures.

Storing EtBr

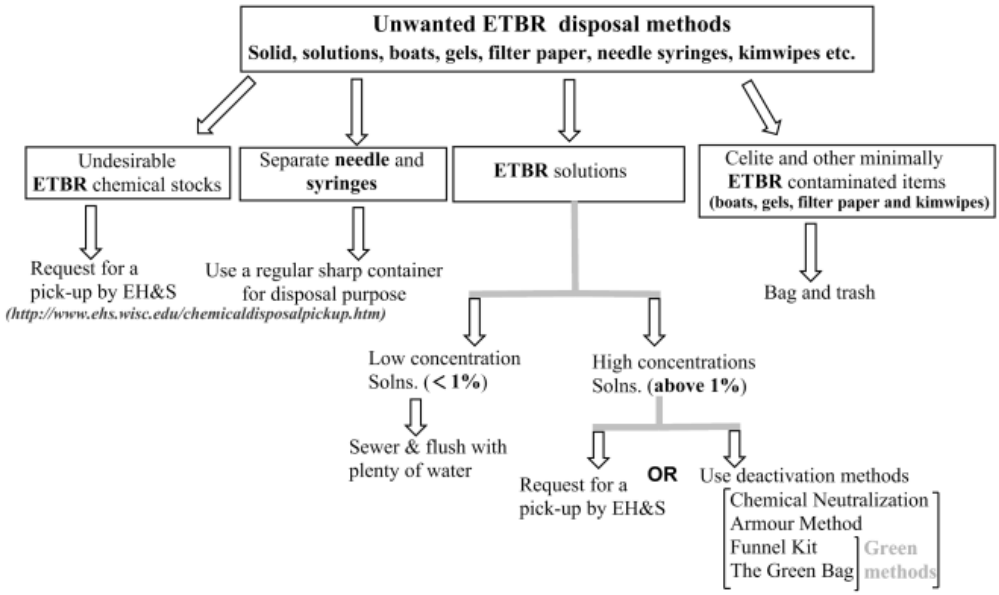
EtBr and EtBr -stock solutions should be stored in a cool, dark, dry place separate from strong oxidizing agents e.g. nitric acid. Stock solution bottles should be of a type that is not easily knocked over and should be kept in a robust liquid proof secondary container when not in use. As with all chemicals, containers should be kept tightly closed and unauthorized access prevented.

Disposal

Disposal of used materials is relatively simple. Materials are separated into 3 key groups:

- Unused powders and stock solution
- Low concentrations of EtBr solutions and EtBr -containing gels
- Minimally contaminated equipment

The chart below provides details on the proper disposal route.



Environmentally Friendly

Laboratories producing large volumes of EtBr containing buffers and solutions should consider the use of commercially available filter cartridges to remove ETBR from buffers and other contaminated solutions prior to discharge to sink. Below are a few examples:

- Whatman™ sells the Extractor® EtBr System – a one-step filtration funnel device for the removal of ethidium bromide from gel-staining solutions. Each device can decontaminate up to 10 liters of gel-staining solution. After filtration, the decontaminated solution can be safely poured down the laboratory drain.
- Amresco makes De-staining Bags while BIO 101 produces the Green Bag® Kit. Another simple charcoal filtration method is the Green Bag, manufactured by BIO 101. These both use small "tea" bags containing activated carbon to remove EtBr from solutions.

NOTE: similar products are available from other vendors

If using any of these products contact EH&S and request a pickup of the used filters.

Alternatives of EtBr/safe staining reagents

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.

SYBR® Safe DNA gel stain is a highly sensitive stain for visualization of DNA in agarose or acrylamide gels. SYBR® Safe stain is specifically formulated to be a less hazardous alternative to EtBr that can be used with either blue-light or UV excitation. SYBR® Safe stain is supplied as either a concentrate or a ready-to-use solution that can be used like an EtBr solution. The stain is also suitable for staining RNA in gels.

GelRed is a sensitive, stable and environmentally safe fluorescent nucleic acid dye designed to replace the highly toxic EtBr for staining dsDNA, ssDNA or RNA in agarose gels or polyacrylamide gels. GelRed is far more sensitive than EtBr without requiring a destaining step.

References and Important Links

The following references and links provide additional information on the hazards associated with EtBr and safe work practices:

1. Sambrook, J.; Fritsch, E. F.; Maniatis, T. Molecular Cloning: A Laboratory Manual, 2nd ed.; Cold Spring Harbor Laboratory Press: Plainview, NY, 1989.
2. Cook, P. R.; Brazell, I. A. Nature 1976, 263, 679–682.
3. Voytas, D. Current Protocols in Molecular Biology 2000, 2.5 A.1-2.5A9.
4. CRC Handbook of Laboratory Safety, 5th ed.; Furr, K. A., Ed.; CRC Press LLC: Boca Raton, FL, 2000.
5. Lunn, G.; Sansone, E. Destruction of Hazardous Chemicals in the Laboratory; John Wiley & Sons, Inc.: New York, 1990.
6. www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/Application_Notes/4549.pdf
7. https://www.lifetechnologies.com/us/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-gel-electrophoresis/dna-stains/sybr-safe.html
8. http://arboretum.harvard.edu/wp-content/uploads/GelRed-stain.pdf
9. http://www.ocs.umich.edu/pdf/GelRedFactSheet.pdf

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