



INDIANA UNIVERSITY

Chemical Fact Sheet

Ethidium Bromide

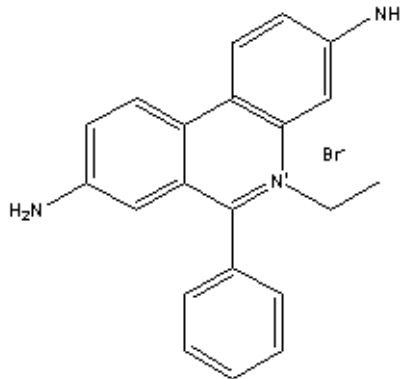


Chemical Identification

Chemical Name: Phenanthridinium, 3,8-diamino-5-ethyl-6-phenyl-, bromide
CAS Number: 1239-45-8
RTECS Number: SF7950000
Molecular Formula: $C_{21}H_{20}BrN_3$
Molecular Weight: 394.35

Synonyms/Trade Names

Homidium bromide
2,7-Diamino-10-ethyl-9-phenylphenanthridinium bromide
3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide
2,7-Diamino-9-phenyl-10-ethylphenanthridinium bromide
2,7-Diamino-9-phenylphenanthridine ethobromide
Dromilac
RD 1572



Use

Ethidium Bromide (EtBr) is commonly used as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of gel-based nucleic acid separation. EtBr is a dark red, crystalline, non-volatile solid, moderately soluble in water, which fluoresces readily with a reddish-brown color when exposed to ultraviolet light (UV). Although it is an effective tool, its hazardous properties require safe handling and disposal procedures.

Hazards

EtBr is a potent mutagen and moderately toxic after an acute exposure. EtBr can be absorbed through the skin, so it is important to avoid any direct contact with the chemical. EtBr is also 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.

Safety Precautions

Consult the Laboratory Chemical Safety Plan (LCSP)¹, read the laboratory chemical safety summary for ethidium bromide found in Appendix D, and read the Material Safety Data Sheet (MSDS) provided from the manufacturer prior to using this chemical.

People using EtBr should follow all safety procedures. Pure EtBr should only be handled in a fume hood with the user wearing protective equipment that includes a lab coat, closed-toe shoes, chemically resistant gloves, and chemical safety goggles (not just safety glasses).

Nitrile is an effective barrier to short-term exposure to EtBr. Users should wash their hands after removing their gloves, even if they are certain the gloves weren't punctured, to remove any residue that may have contacted the skin.

An emergency eyewash and shower should be accessible nearby. Like other toxics, EtBr should be used in a specially designated area where no eating or drinking is allowed. When using ultraviolet light to visualize EtBr, the user must wear UV blocking eyewear or work in a UV cabinet with shielding glass in place.

Alternative fluorescent dyes have been developed that manufacturers claim are less toxic and can detect nucleic acid components at lower concentrations than EtBr. They must still be handled and disposed of properly.

**Emergency Exposure Procedures**

If EtBr contacts the eyes, immediately flush them with copious amounts of cold water for at least 15 minutes. For skin contact, immediately wash the affected area with soap and copious amounts of cold or cool water. If a person inhales EtBr dust, move them to an area where they can breathe fresh air. After any exposure, refer to the instructions for medical consultation in the Laboratory Chemical Safety Plan (LCSP) and be sure to provide the MSDS to the attending physician.

Spill Response Procedures

Be prepared to safely remediate small spills of EtBr by having a spill kit on hand at all times, understanding the hazards, and using proper personal protective equipment (gloves, safety glasses, lab coat, etc.).

Large Spill

Notify all others in the room that the spill has occurred. Evacuate the room or immediate area and call University Office of Environmental, Health, and Safety Management (UOEHSM) for assistance with the cleanup. Post a sign warning others not to enter the area. Provide any assistance and information you can to the spill responders.

If a large spill of EtBr enters a sink or floor drain contact the UOEHSM (855-6311) during normal business hours. After hours contact the IU Police Department (855-4111).

Small Spill

Always wear full protective clothing, as described above, during any cleanup procedure. You should only clean up spills if you are aware of the hazards and have the proper safety and cleanup equipment. Contact UOEHSM if you need assistance.

Use UV light to locate the spill; EtBr's fluorescence is easy to see. If the spill is powder, carefully wipe it up with wet paper towels and follow the decontamination procedure below. If the EtBr spill is liquid, absorb freestanding liquid with dry paper towels. Use a UV light source and illuminate the area to locate any remaining EtBr. Then use the decontamination procedure. After the decontamination procedure, re-survey the area with UV light to ensure that all the EtBr has been collected.

Surface Decontamination²

Prepare the decontamination solution just prior to use. Since it is acidic, wear full protective equipment (lab coat, gloves, and goggles, not safety glasses) when preparing or using the decontamination solution. The solution consists of 4.2g of sodium nitrite, NaNO₂ (CAS #7362-00-0), and 20 ml of 50% hypophosphorous acid solution, H₃PO₂ (CAS #6303-21-5), in 300 ml of water.

Procedure:

- 1) Wash the area with a paper towel soaked in decontamination solution. Then rinse the area five times with paper towels soaked with tap water, using a fresh towel each time.
- 2) Soak all the towels in decontamination solution for one hour. Then remove them, gently wring out excess solution, and dispose of as dry waste in a separate bag along with the contaminated gloves.
- 3) Using a UV light, check the area to ensure that all the EtBr has been removed.
- 4) Bottle and label the decontamination solution. Contaminated solid waste (towels and gloves) should be bagged and labeled. Bring the solution and solid waste to UOEHSM Open House or call to schedule a pick up.
- 5) If the acid may damage the contaminated surface, use a few additional rinses. Soak all the towels in decontamination solution for at least one hour before disposal.



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Disposal Procedures

Ethidium bromide gels and unwanted solid EtBr must be disposed through UOEHSM. Liquids or working solutions may also be disposed through UOEHSM or rendered non-toxic using one of the two methods described below as the last step in your experimental procedure.

UOEHSM Disposal

Gels may be accumulated in one gallon plastic jars or five gallon buckets. Do not mix other waste with gels such as plastic wrap, gloves, paper towels, sharps, or other contaminated items. Any additional materials in the gels must be removed by waste handling personnel and may result in injury. These solid materials must be packaged separately and labeled as chemically contaminated items (CCI). Sharps must be in a puncture resistant container and labeled appropriately. Label all waste with a Hazardous Chemical Waste tag and call UOEHSM (855-6311) to have the containers picked up for disposal.

HAZARDOUS CHEMICAL WASTE TAG
Use this tag for all hazardous waste.

UNIVERSITY OF INDIANA
10376

Name: _____
Your Name: _____
Your Group/Project: _____
Container Size: _____

DATE: _____

COMPLETE PHYSICAL COMPOSITION: List % of amount of each constituent including water (where applicable):

Constituent	%	Other

Check for volatility: _____
 Flammable: _____
 Corrosive: _____
 Oxidizing: _____
 Toxic: _____
 Hazardous: _____

Location: _____
 Quantity: _____
 Date: _____

NOTES: See University of Indiana Environmental Health and Safety Management Office website for more information regarding hazardous waste disposal procedures. For more information, contact the Environmental Health and Safety Office at (812) 855-6311.

- All waste that is placed into this tag must be properly labeled, stored, and disposed of according to the instructions on this tag. The container will remain at the University of Indiana until the waste is disposed of. The waste will be disposed of at the University of Indiana's waste disposal facility.
- All hazardous waste must be properly labeled, stored, and disposed of according to the instructions on this tag.
- Substances are stored in sealed containers. Seal each chemical container. Record the appropriate MSDS (including hazard labels).
- Accumulate solids (gloves, paper, paper towels, etc.) in separate containers from liquid waste.
- Use secondary containment.
- Each container must be properly sealed and labeled with a hazard label.
- Do not put liquid waste in plastic containers as they are to be upgraded and used.
- Waste handling will be done at the University of Indiana's waste disposal facility.
- Storage of waste containers: closed.

Ethidium Bromide Detoxification

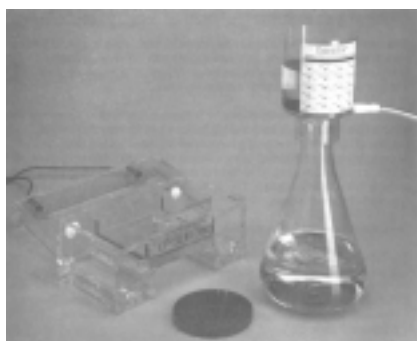
Disposal of EtBr solutions into the sanitary sewer or sink drain is not permitted. Liquids containing EtBr may be given to UOEHSM for disposal or detoxified in the laboratory using one of two general methods: (1) extraction/adsorption, and (2) chemical degradation. If you choose to detoxify liquid EtBr in your laboratory the process must meet all the following conditions.

- The material should contain EtBr and non-hazardous constituents. If any other hazardous constituents are present then label the waste and deliver to UOEHSM or call to schedule a pick up.
- The procedure must be performed as close as practical to the location where the EtBr was used.
- The amount of EtBr to be rendered non-toxic in a single batch does not exceed the quantity specified in the procedure or the capacity of the equipment or filters.
- The procedure must comply with the requirements for disposal of materials resulting from the detoxification process.
- The procedure must be performed by someone trained in handling and treatment of EtBr, its waste, and responding to spills or emergency situations. The training must be documented using the form provided in the LCSP.
- The user must follow the guidelines for handling ethidium bromide found in the Laboratory Chemical Safety Summaries (Appendix C of the LCSP) and the General Procedures for Handling Hazardous Chemicals (Chapter 5) described in the National Research Council's report, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*³, or in other peer-reviewed scientific journals.



Ethidium Bromide Extraction

Extraction is the simpler method and requires setting up a granular charcoal filtration system or using devices such as the EXTRACTOR[®] from Schleicher & Schuell or similar products from other sources. It is important not to exceed the extractor's capacity, as described in the product's instructions. Prior to drain-disposing the effluent from the extractor, you must ensure that EtBr has been removed by checking it for fluorescence using a UV light. When exhausted, the extractor and/or filter media must be discarded through UOEHSM.



Ethidium Bromide Degradation⁴

If extraction is not possible the following EtBr degradation procedure can be used. This procedure is more complicated and should be performed in a fume hood. Contact UOEHSM for additional information regarding this procedure.

Procedure:

- 1) Dilute solutions of EtBr to a final concentration of less than or equal to 0.034% w/v (34mg EtBr/100 ml solution).
- 2) Add 10 ml fresh bleach for every 1 mg EtBr (bleach deteriorates upon exposure to air).
- 3) Stir the mix continuously for four hours or overnight.
- 4) Test the final solution with a UV light to ascertain that the EtBr is destroyed.
- 5) Of the final solution, drain-dispose one part solution with 20 parts tap water.

References

- ¹ Indiana University, 1996, *Laboratory Chemical Safety Plan*, appendix C.
- ² Lunn, G. & Sansone, E.B., 1990, *Destruction of Hazardous Chemicals in the Laboratory*, John Wiley and Sons, New York, 1990, p. 119-120.
- ³ National Research Council, 1995, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*, National Academy Press, p. 79-105, 310.
- ⁴ Kaufman, J.A., ed., 1990, *Waste Disposal in Academic Institutions*, Lewis Publishers, p. 127.