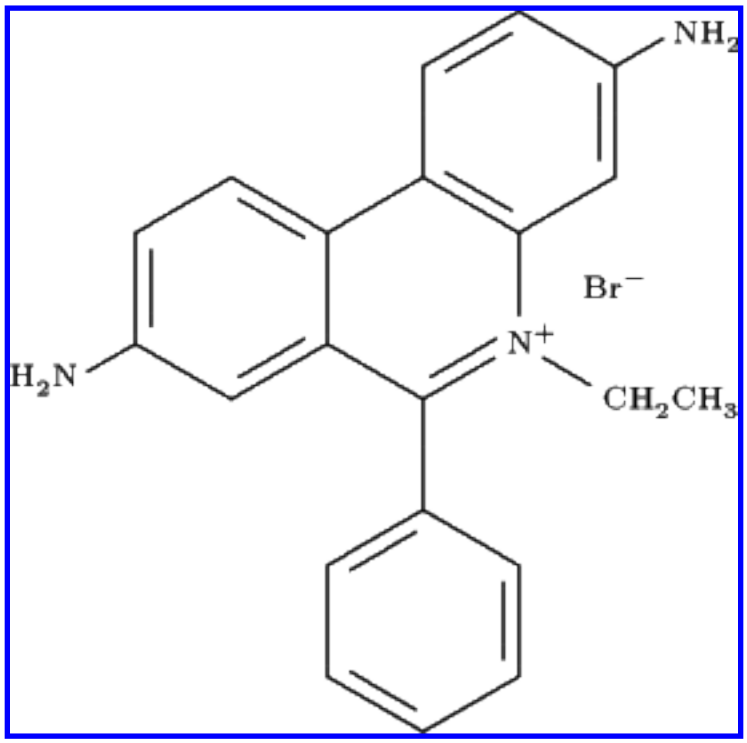
Ethidium bromide

Ethidium bromide (EtBr) is an intercalating agent commonly used as a nucleic acid stain in molecular biology laboratories for techniques such as agarose gel electrophoresis. When exposed to ultraviolet light, it will fluoresce a red-orange color, especially when it binds to DNA (30-fold increase). This is likely due to rigid stabilization of the phenyl moiety. Ethidium bromide is a very strong mutagen, and may possibly be a carcinogen or teratogen. Important hazard information follows. As with most fluorescent compounds, it is aromatic. The main portion of the molecule is a tricyclic structure with aniline (amino-benzene) groups on either side of a pyridine (six-atom, nitrogen-containing, aromatic ring).



Hazards

Ethidium bromide is strongly mutagenic and is irritating to the eyes, skin, mucous membranes and upper respiratory tract. The health effects of ethidium bromide exposure have not been thoroughly investigated. It is suspected to be carcinogenic and teratogenic because of its mutagenicity, although there is no direct evidence of either effect. The toxic effects of ethidium bromide may be experienced if swallowed, inhaled or absorbed through the skin.

Safe Handling

Preparation of stock solutions and any operations capable of generating ethidium bromide dust or aerosols should be conducted in a fume hood to prevent inhalation. Gloves should be worn at all times. Latex gloves offer little protection from ethidium bromide -- nitrile gloves are much more effective. When working with high concentrations or for a prolonged period of time, double gloving can further reduce the risk of exposure, especially if the outer glove is replaced whenever significantly contaminated.

Spills or Personal Exposure

As with any chemical, if spilled on skin or eyes, rinse for 15 minutes using a safety shower or eyewash. If inhaled or swallowed, seek medical attention immediately.

For spills, use a spill pillow or absorbent to soak up aqueous ethidium bromide. Carefully clean up solid ethidium bromide to avoid creating dusts. Place in a sealable container and dispose in a medical waste box.

Waste Disposal

Electrophoresis Gels

Trace amounts of ethidium bromide in gels should not pose a hazard. Higher concentrations, e.g., when the color of the gel is dark pink or red, should not be placed in laboratory trash. EHS recommends the following:

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   * Less than 0.1% ethidium bromide:  place in laboratory trash
   * More than or equal to 0.1%:  place in biohazard box for
incineration.
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Ethidium Bromide Solutions

Solutions containing ethidium bromide should be deactivated, neutralized and poured down the drain with copious amounts of water. Deactivation may be confirmed using UV light to detect fluorescence. There are three recognized methods for deactivation:

Armour Method

This is the simplest method, but is somewhat controversial. One study found traces of mutagenic reaction mixtures using this method. (Lunn, G. and E. Sansone, Analytical Biochemistry, vol. 162, pp. 453-458, 1987) 1. Combine equal amounts of ethidium bromide solution and household bleach. 2. Stir constantly for four hours or let sit for 2-3 days. 3. Adjust pH to 4-9 with sodium hydroxide. 4. Pour down drain with copious amounts of water.

Lunn and Sansone Method

For each 100 ml of ethidium bromide solution: 1. Add 5% hypophosphorus acid. 2. Add 12 ml of 0.5 M sodium nitrate. 3. Stir briefly and let stand for 20 hours. 4. Adjust pH to 4-9 using sodium hydroxide. 5. Pour down drain with copious amounts of water.

Quillardet and Hoffnung Method

This method uses 0.5 M potassium permanganate and 2.5 M hydrochloric acid. Since chlorine gas may be released in significant concentration, EHS does not recommend using this method.

Charcoal Filtration

Filtering the aqueous ethidium bromide waste solutions, free of other contaminants, through a bed of activated charcoal is a relatively simple and effective method for removal of ethidium bromide. Schleicher and Schuell (603-352-3810 or http://www.s-and-s.com) supply commercial filter funnel kits that use packaged charcoal disks that are graduated for easily tracking the amount of aqueous solution calculated for a fixed quantities of ethidium bromide residue.

1. Filter the ethidium bromide solution through charcoal filter. 2. Pour filtrate down the drain. 3. Place charcoal filter in a sealed bag (e.g., Zip-loc) and place in biohazardous waste box for incineration.

Gloves, Equipment and Debris Gloves, test tubes, paper towels, etc., that are grossly contaminated with ethidium bromide should be placed in medical waste for incineration. Consider deactivating in bleach before disposal if the items are significantly contaminated.

Other Names

Ethidium bromide is also known as:

- 2,7-diamino-N-ethyl-6-phenyl-phenanthridinium bromide
- 2,7-diamino-10-ethyl-6-phenyl phenanthridinium bromide
- 2,7-diamino-10-ethyl-9-phenyl phenanthridinium bromide. FW 394.3, C21H20N3Br.

Note: hazard information was copied verbatim from http://www.princeton.edu/~ehs/wastepaper/99-4.html

de:Ethidiumbromid

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