

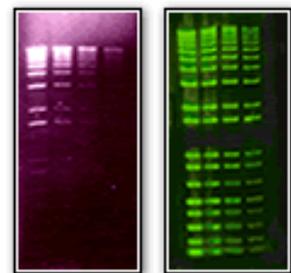
Ethidium Bromide: **Alternatives & Disposal Methods**

I. Overview

Ethidium bromide and similar fluorescent compounds such as Acridine Orange are normally used to visualize DNA on a gel. It fluoresces under ultraviolet light, especially when bound to double-stranded DNA. **Unfortunately, ethidium bromide and its breakdown products are potent mutagens and carcinogens.** Because of its mutagenic and carcinogenic properties, drain disposal of higher concentrations of ethidium bromide is not permitted at UIC. Ethidium bromide is available as a powder, a concentrated solution (10 µg/ml or 10 ppm) and as a dilute solution. Researchers may find that the less hazardous alternatives to ethidium bromide are easier and safer to manage.

II. Alternatives to Ethidium Bromide

GelRed and GelGreen are nucleic acid gel stains from the company Biotium which offer cell membrane impermeability, high sensitivity, instrument compatibility, stability, and compatibility with all downstream manipulations. Concentrations less than 750 mg/L (750 ppm) may be disposed of in the sink if they have been neutralized with sodium bicarbonate first. Biotium, the manufacturer of GelRed and GelGreen produced a safety report and an overview of the dye, which can be viewed [here](#). Visit Biotium's website to learn more http://www.biotium.com/product/product_info/Newproduct/GelStains.asp

EB
Prestain
StainingGelGreen™
Post
staining

SYBR Safe comes from the company Invitrogen and claims that Sybr safe is less mutagenic, non genotoxic and non-hazardous for waste disposal. Full details of this product including a downloadable version of a report, compiled by Molecular Probes, on the mutagenicity and [environmental safety](#) of the product, from the test results of two independent organizations, can be accessed at: <http://probes.invitrogen.com/products/sybrsafe/>. SYBR Safe in particular might be less mutagenic than ethidium bromide, but should be treated like ethidium bromide before disposal.

These next few nucleic acid dyes and stains described below are also mutagens and possible carcinogens. They should be treated like ethidium bromide with the methods described below, under "Disposal Procedures for Ethidium Bromide".

Crystal violet binds to DNA in a similar way to ethidium bromide and although it is a mutagen, it is not thought to be as harmful as ethidium bromide. Because it can be viewed in normal daylight (avoiding the need for damaging UV light), some researchers have advocated its use where functional DNA is to be recovered from a gel.

Methylene blue (and its oxidation products such as **Azures A, B, and C, Toluidine blue O, Thionin** and **Brilliant cresyl blue**) are used individually or as mixtures (often in proprietary formulations). Although their exact mode of action is unknown, they are thought to bind ionically to the outside of nucleic acids (to the negatively-charged phosphate groups) and can therefore be used to detect both DNA and single-stranded RNA.

Megafluor is a fluorescent stain for detection of double stranded DNA after agarose gel electrophoresis. The detection limit is said to be in the low picograms (about 3-5pg) and since MegaFluor is only added to the sample and not to the gel nor to the buffer, there is no background in the gel. This leads to a consistent increase of the exposure time when photographing the gel (up to 30 seconds) and therefore to a significant increase of the detection limit, Megafluor can consistently differentiate between strands with just 2% difference in base pairs.

III. Disposal Procedures for Ethidium Bromide

Part I- Electrophoresis Gels

Trace amounts of ethidium bromide in gels should not pose a hazard. Gels that contain ethidium bromide may be doubled-bagged, labeled "non-hazardous" and placed in the trash if they contain less than 0.1% (1000 ppm or 1000ug/mL) ethidium bromide. Higher concentrations, e.g., when the color of the gel is dark pink or red, should be treated as biohazardous waste. Please contact the following individuals for questions regarding biohazardous waste removal:

Hospital & Clinics
Hospital Environmental
Services
6-3688

**College of Dentistry
(BLDG 940)**
Fred Chappa
6-7633

MBRB (BLDG 919)
Bernie Greski
6-6963

BRL (BLDG 932)
Scott Hauff
6-7052

SPHW (BLDG 930)
Margit Javor
3-1390

All East Side Buildings
Building Services
6-1799

COMRB (BLDG 934)
Building Services
6-7052
OR
Anthony Capistran
3-1551

**All Other West Side
Buildings**
Building Services
6-7468

Part II- Ethidium Bromide Solutions

- Aqueous solutions containing <math><10\text{ug/ml}</math> (10 ppm or 0.001%) ethidium bromide can be released to the drain.
- Aqueous solutions containing >10ug/ml ethidium bromide should be filtered or deactivated using one of the methods described below. EHSO strongly recommends **charcoal filtration** over chemical deactivation.
- Solutions containing heavy metals, organics, cyanides or sulfides should be disposed as hazardous waste. Email chemwaste@uic.edu to submit an [Unwanted Chemical Removal Form](#).

A. 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. The filtrate may be poured down the drain.

There are three simple kits available for charcoal filtration:

1. Funnel Kit

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 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.



2. The Green Bag for dilute (10ug/L) solutions

Another simple charcoal filtration method is the **EtBr GreenBag™ Disposal Kit**, manufactured by MP Biomedicals. 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). The



treated water can be disposed of in the sink. The tea bags, which contain activated carbon, absorb the ethidium bromide so tightly that it is safe to put them in the trash. Green Bags are available through [MP Biomedicals \(www.mpbio.com\)](http://www.mpbio.com)

3. 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.

Part III- Gloves, Equipment and Debris

Clean spills of ethidium bromide carefully with soap and water. Collect the soap, water and ethidium bromide mixture onto absorbent material. If concentrated or powdered ethidium bromide was spilled, place the spill cleanup materials in a sturdy screw top container and dispose of as biohazardous waste (see Disposal Procedures for Ethidium Bromide, Part I- Electrophoresis Gels). All other materials may go in the trash after it has been double bagged.



Items contaminated with concentrated ethidium bromide are managed as biohazardous waste. Gloves, test tubes, paper towels, etc., that are grossly contaminated with ethidium bromide should be placed into a red biohazardous waste bag for pick up. Consider deactivating in bleach before disposal if the items are significantly contaminated.

Other items contaminated with dilute ethidium bromide may be double-bagged and placed in the trash. Please double-bag to prevent any ethidium bromide leaks. Glass pipette tips contaminated with dilute ethidium bromide are dried and placed into a broken glass container.



This information of Ethidium Bromide disposal was created by UIC, [Princeton University](#), and the [University of Washington](#).