Ethidium bromide decontamination and remediation of electrophoresis buffers

Created: Mar 02, 2007; **Last modified**: May 17, 2017, **Version:** 2.0

Ethidium bromide (3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide, $C_{21}H_{20}BrN_3$) is an intercalating agent used as a fluorescent tag in nucleic acid based molecular biology techniques such as agarose gel electrophoresis. It is commonly abbreviated as "EtBr", which is erroneous as this is the correct abbreviation for bromoethane. When exposed to ultraviolet light, it will fluoresce with an orange colour, intensifying almost 20-fold after binding to DNA. It has also been used extensively with the name homidium since the 1950s in veterinary medicine to treat trypanosomiasis in cattle. Ethidium bromide may be a mutagen, although this depends on the organism exposed and the circumstances of exposure. DNA can be photographed in agarose gels stained with ethidium bromide by illumination with UV light (>2500 µW/cm2). A UV transilluminator is typically used for this purpose, and commercial models are available designed specifically for DNA visualization and photography (e.g., UVP, Bio-Rad, Stratagene). To facilitate visualization of DNA fragments during the run, ethidium bromide solution can be added to the electrophoresis buffer to a final concentration of 0.5 µg/ml. As our laboratory uses large format (25 x25 cm) agarose electrophoresis gels and a total tank volume of up to 2 litres, it is of CRITICAL IMPORTANCE that all electrophoresis buffers be decontaminated for ethidium bromide prior to flushing them down the drain. It is VITAL that molecular laboratory labs maintain as low as possible environmental impact on their surroundings!

Decontamination procedure

1. Label a 20 litre plastic jerry can with a label clearly indicating the nature of its contents:

2. Place 60 grams of activated charcoal (300 mg per 100 mL) inside the plastic jerry can (which at this stage should be empty).

3. As buffer is exhausted after several electrophoretic runs (normally between 4 and 5), take a large 200 mL surgical syringe or vacuum pump set-up with tandem traps using Kitasato flasks and remove used buffer.

4. Pour used buffer inside the plastic jerry can with activated charcoal and gently swirl can to maximize exposure of activated charcoal to buffer.

5. After 48 hours, most ethidium bromide will have been absorbed by the activated charcoal and the buffer will have been decontaminated.

6. Nevertheless, accumulate buffer in jerry can until the 20-litre mark has been reached. Allow jerry can to rest with buffer for at least 1 week to ensure proper decontamination of all contents.

7. Pour decontaminated buffer from jerry can and into drain without allowing the activated charcoal to flow out.

8. Recollect remaining activated charcoal and place in large zip bag or suitable plastic container.

9. Label container with ethidium bromide contaminated charcoal as ENVIRONMENTAL HAZARD and turn in for proper disposal.

10. Refill plastic jerry can with 60 gram of activated charcoal and continue decontamination process of all electrophoresis buffers.

Notes

1. CAUTION: Ethidium bromide is a mutagen and potential carcinogen. Gloves should be worn and care should be taken when handling ethidium bromide solutions.

2. Prolonged exposure of the DNA‑ethidium bromide complex to UV light will cause damage to the DNA and should be avoided.

3. Gels that have been run in the absence of ethidium bromide can be stained by covering the gel in a dilute solution of ethidium bromide (0.5 µg/ml in water) and gently agitating for 10 to 30 min.

4. If necessary, gels can be destained by shaking in water for an additional 30 min. This serves to remove excess ethidium bromide, which causes background fluorescence and makes visualization of small quantities of DNA difficult.

References

1. Wikipedia contributors. Ethidium Bromide. Wikipedia, The Free Encyclopaedia. Jun 08, 2008, at 04:15 UTC. Available at: http://en.wikipedia.org/wiki/Ethidium Bromide.

2. Current Protocols in Molecular Biology, Chapter 2.5 A9, Frederick M. Ausubel et al. 2003.

3. Bensaude, O. Ethidium Bromide and Safety- Readers Suggest Alternative Solutions. Trends Genet. 4:89; 1988.

4. Lunn, G.; Sansone, E.B. Decontamination of Ethidium Bromide Spills. Appl. Ind. Hyg. 4: 234-237; 1989.

Revision history

1. Original document.

2. Formatting changes and minor revision.