

Protocol For The Destruction of Ethidium Bromide.

Introduction

Ethidium bromide liquid and solid waste presents a disposal dilemma. It is not regulated as a hazardous waste by the EPA but because of its toxicity it should not be disposed of as normal solid waste in the municipal waste stream. The Environmental Health and Safety Department has determined that chemical destruction of this material by training researchers within individual laboratories is the preferred treatment method. The procedure outlined in the following protocol is a proven method for total destruction of the mutagenic potential of this material.

Destruction and Decontamination Procedures

Laboratory Safety Practices and Equipment

1. Prepare decontamination solution in a fume hood as the decontamination solution gives off a small amount of nitrogen dioxide, a toxic gas, when initially mixed
2. Wear proper PPEs including but not limited to gloves and safety glasses

Preparation of Decontamination Solution

1. Add 20ml of hypo phosphorous acid (50%) to a solution of 4.2g of sodium nitrite in 300ml water
2. Stir briefly
3. Prepare the solution the day of use

Destruction of Ethidium Bromide (EB) Solutions*

1. For EB solutions with concentration of EB > 0.5mg/ml, dilute the EB solution with water to 0.5mg/ml
2. Add decontamination solution to EB solution to final concentration of 25% decontamination solution (i.e. add 25ml decon solution to 75ml EB solution)
3. Stir briefly and hold for 20 hours
4. Test mixture for fluorescence; repeat procedure with fresh decon solution if fluorescence is present
5. Neutralize the solution (pH between 5 and 9) with sodium bicarbonate and drain dispose the material

*This procedure has been validated for solutions of EB (0.5mg/ml) in water, TBE buffer, Mops buffer and cesium chloride gradients

Decontamination of Surfaces contaminated with EB

1. Soak a paper towel in decon solution, place on contaminated surface and scrub
2. Scrub another five times with paper towels soaked in water, using a fresh towel each time
3. Place all towels in a large container and soak in fresh decontamination solution for one hour
4. Test squeeziings from final towel scrub and mixture for fluorescence; repeat procedure with fresh decon solution if fluorescence is present
5. Neutralize the solution (pH between 5 and 9) with sodium bicarbonate and drain dispose the material

Note: Solid materials contaminated with EB such as pipette tips and gels can also be decontaminated using the decon solution. Such materials can be placed in a bucket or similar container with the decon solution and solution checked for fluorescence. The decontamination procedure will only work however if the solids are all able to come into contact with the decon solution. An alternative is to request the Environmental Health and Safety Department to pick up the solid waste.

References

1. Lunn, G. and Sansone, EB. 1987. Ethidium Bromide: destruction and decontamination of solution. *Anal Biochem* 162:453
2. Lunn, G. and Sansone, EB. 1989. Decontamination of ethidium bromide spills. *Appl Ind Hyg* 4:234