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PROTOCOL FOR THE DESTRUCTION OF ETHIDIUM BROMIDE USED IN MOLECULAR BIOLOGY

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Abstract- A rapid method for the destruction of ethidium bromide has recently been published. A protocol for its use is described.

Key Word Index- Ethidium bromide, destruction, mutagens

Introduction

Ethidium bromide (EB) is widely used for the visualization of DNA, particularly in gel electrophoresis. It is, however, a known mutagen and as such must be handled with care and properly disposed. Recently, Lunn and Sansone (1987) performed a series of experiments on methods for the destruction of the mutagenic properties of ethidium bromide. In addition, they assayed the mutagenic activities of the reaction products. We offer this note only in the interest of the wider distribution of their original research. The interested reader is referred to Lunn and Sansone (1987) for additional details.

Protocol: Destruction of ethidium bromide.
To destroy 100 ml of ethidium bromide solution (@ 0.5mg/ml),

1. Prepare a fresh 5% solution of hypophosphorous acid (HPPA). Add 10 ml of HPPA (cf. Fisher A154-500, 50% hypophosphorous acid) to 90 ml water to produce a 100 ml of 5% solution (v/v).
2. Prepare a fresh 0.5M sodium nitrite (SN) solution: to 3.45 g of sodium nitrite (cf. Sigma S-2252), add water to 100 ml.
3. Add 20 ml of 5% solution of HPPA per 100 ml of ethidium bromide solution.
4. Check to see that pH is less than 3.0. If it is greater than 3.0, add HPPA until pH is less than 3.0. Note: a low pH is critical for a complete reaction!
5. Add 12 ml of 0.5M sodium nitrite solution.
6. Stir briefly.
7. Let stand in a hood for 20 hours.
8. Add sodium bicarbonate until the solution is neutral (test with pH paper).
9. Check for no fluorescence, pour solution down the drain.

This method destroys ethidium bromide without producing mutagenic products. The current practice of using bleach (ex. Chlorox, sodium hypochlorite) should not be used for the destruction of ethidium bromide because it produces mutagenic products. The aforementioned protocol was successfully used to destroy ethidium bromide in water, TBE buffer, Mops buffer and cesium chloride solutions (Lunn and Sansone, 1987).

One should note that this protocol is to destroy high concentrations of EB (0.5mg/ml) but one should not reduce the concentrations of HPPA and SN when treating typical concentrations of 0.5 ug/ml EB solutions. The high

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concentrations of HPPA and SN drive the reaction to completion to remove essentially all of the EB in 20 hours. Rather than disposing of ethidium bromide waster solutions daily, we have found it much more economical to accumulate all ethidium bromide solutions during the week and then prepare just enough of the 5% HPPA and sodium nitrite solutions on Friday to treat all our waste ethidium bromide solutions. We leave the material in the hood over the weekend and pour it out on Monday.

Literature Cited:

Lunn, G. and E.B. Sansone. 1987. Ethidium bromide: destruction and decontamination of solutions. Anal. Biochem. 162: 453-458.