Preparation of ethidium bromide for visualizing PCR products

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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. Erroneously abbreviated as "EtBr", as this actually corresponds to bromoethane. It was used extensively with the name homidium during the 1950s in veterinary medicine to treat cattle trypanosomiasis (as it arrests kinetoplastoid replication by changing the conformation of DNA to Z-DNA). When exposed to ultraviolet light (the absorption maxima is between 210 - 285 nm) it will fluoresce with an orange to reddish-brown colour (605 nm) which intensifies nearly 20-fold after binding DNA. DNA can be photographed in agarose gels stained with ethidium bromide by illumination with UV light (>2500 μW/cm2). Ethidium bromide binds DNA, which is why it is considered highly toxic, mutagenic, and potentially carcinogenic or teratogenic (although there is no scientific evidence of these effects). Exposure to ethidium bromide can occur through inhalation, ingestion, or skin absorption. Acute ethidium bromide poisoning is characterized by irritation of the mouth, upper respiratory tract, skin, and eyes. The use of nitrile gloves is mandatory during preparation and handling as they provide greater resistance than latex. To visualize DNA fragments on agarose gel, ethidium bromide can be added to the electrophoresis buffer at a final concentration of 0.5 μg/ml. As our laboratory uses large format agarose electrophoresis gels (25 x25 cm) and a total tank volume of 2 liters, it is EXTREMELY IMPORTANT that all electrophoresis buffers be decontaminated with activated carbon before being discarded down the drain. Filtering ethidium bromide-stained aqueous solutions (electrophoresis buffer) through activated carbon is a simple and effective decontamination method. Once filtered, the buffer can be poured down the drain while the activated carbon is eliminated as toxic waste. This reduces the weight and volume of waste that should continue to be considered toxic (activated carbon).

Procedure

1. Don proper personal protective equipment including buttoned lab coat, nitrile gloves, N95 respirator and goggles.

2. Retrieve powdered ethidium bromide bottle (Sigma E7637-1G) from the blue chemical cupboard located in the storage room.

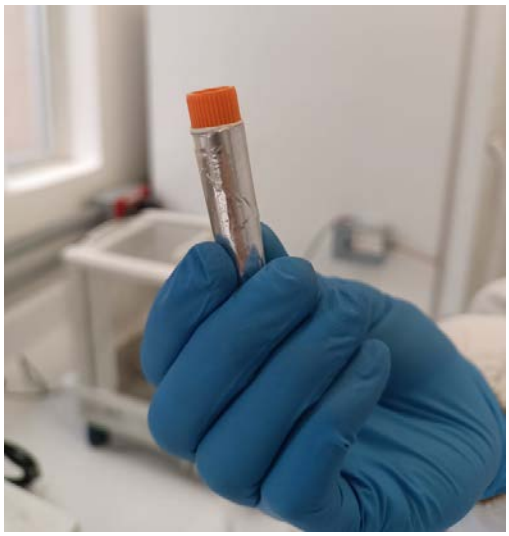
3. Weigh 10 mg of powdered ethidium bromide in the Ohaus Adventurer balance located in the molecular biology lab.

4. Place the 10 mg of ethidium bromide in a 1.8 ml cryovial.

5. Add 1 ml of dH_20, cap and vortex vigorously for 5 minutes.

6. Place self-adhesive aluminium foil tape around cryovial to protect from light (see below).

7. Add self-adhesive label over foil with the following legend "*Ethidium Bromide 10 mg/ml TOXIC*".



8. Place the foil protected cryovial in a box also protected from light by applying the same self-adhesive aluminium foil tape (see below).

9. Add 5 µl of this 10 mg/ml solution per 100 ml of gel or electrophoresis buffer.

Notes

1. Follow the following protocol for ethidium bromide decontamination of electrophoresis buffers (http://www.genomica.uaslp.mx/Protocolos/Gral_EtBr_Decon_ENG.pdf).

References

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2. Borst P (November 2005). "Ethidium DNA agarose gel electrophoresis: how it started". IUBMB Life. 57 (11): 745–747. https://iubmb.onlinelibrary.wiley.com/doi/pdf/10.1080/15216540500380855

3. Olmsted J, Kearns DR (August 1977). "Mechanism of ethidium bromide fluorescence enhancement on binding to nucleic acids". Biochemistry. 16 (16): 3647–3654 https://pubs.acs.org/doi/epdf/10.1021/bi00635a022

Revision history

1.0 Original document.