Standard Operating Procedures (SOP) for Preparation and Storage of Tribromoethanol (Avertin) – Supplement to University Standard for Preparation, Storage, and Use of Tribromoethanol (Avertin)

Preparation

- Components
    - 2,2,2-tribromoethanol (Sigma-Aldrich T48402 or equivalent)
    - tertiary-amyl alcohol (Sigma-Aldrich 240486 or equivalent); also known as 2-Methyl-2-butanol or amylene hydrate
    - Diluent, usually tissue-culture grade distilled water or injection quality sterile saline
    - Polypropylene is recommended for pipetting the tertiary-amyl alcohol, not polystyrene. Polystyrene can dissolve in the solvent and then precipitate out in the Avertin working solution, possibly causing adverse effects.
- Stock solution (100%)
    - Prepare the stock solution in a chemical fume hood adhering to laboratory safety precautions.
    - Add 2,2,2-tribromoethanol to tertiary-amyl alcohol in a 1g/1ml ratio to a clean container.
    - Dissolve by heating to 40-50°C and stirring WHILE PROTECTED FROM LIGHT.
    - Label the bottle (stock solution) with the reagent name, date of preparation and a use-by date. Aliquot the stock solution into multiple, smaller containers to reduce the risk of contamination due to multiple draws from the same container.
- Working solution
    - Dilute stock solution with warm sterile diluent [physiologic saline (0.9% NaCl) or distilled water (tissue culture or mouse embryo culture grade)]
    - Make 1:80 dilution of stock for a 1.25% working solution (12.5 mg/ml)
        - Example: add 0.5 ml stock to 39.5 mls diluent.
    - Make 1:40 dilution for a 2.5% working solution
    - Filter through 0.20 micron filter
- Ready to use working solution
    - Heat 200 ml embryo quality water (Sigma W1503) to ~ 50^{o}C, set aside
    - Heat 5 ml tertiary-amyl alcohol to ~ 50^{o}C, add 2.5g TBE, swirl to mix/dissolve
    - Add pre-heated water, making sure that final solution doesn't go above 35-40^{o}C
    - Add stir bar to mix well WHILE PROTECTED FROM LIGHT
    - Filter through 0.20 micron filter
    - Place in autoclaved amber bottle or glass bottle wrapped in aluminum foil
    - Label bottle (as 1.25% Tribromoethanol) and store in refrigerator @ 2-8^{o}C

Storage and Expiration Period

- All storage containers should be labelled as Tribromoethanol with the concentration and dates of preparation and expiration
- Tribromoethanol working solution must be stored at 2-8°C in light protected containers.
    - When stored at this temperature, the solution may be used for up to 3 months.
    - Stored Tribromoethanol working solution should be discarded if it becomes discolored (dark yellow or brown)

- Tribromoethanol stock solution should be aliquoted into light protected containers. Frozen aliquots can be stored in a non-cycling freezer for up to 1 year. After 1 year, any unused solution should be appropriately discarded.
- Stock and working solutions should be accessed in a way that maintains their sterility.
- Store the stock and working solutions away from light and tightly capped, as the solution is photosensitive and hygroscopic (e.g., wrap container in aluminum foil)
- A yellow discoloration indicates the presence of toxic products and the stock solution must be discarded, even if it is before the use-by date.