Technical Data Sheet

Gach : Glutaraldehyde - Carbohydrazide

#15920

This water miscible (aqueous) embedding medium prevents the loss of lipids caused by organic solvents and also the clouding of tissue observed during specific reactions involving the use of metallic fixatives (e.g., OsO4 and Potassium Permanganate).

The GACH embedding medium is prepared by adding Carbohydrazide to 50% Glutaraldehyde in a final concentration of 150 mg/ml, (e.g., 1.5g of Carbohydrazide to 10ml of 50% Glutaraldehyde).

The mixture is carried out in a pre-cooled beaker kept in an ice bath, using a magnetic stirrer. The Carbohydrazide is added in three portions, and stirred rapidly for 15 minutes after each of the additions.

It can be employed at neutral pH and cured at 37°C. The stock solution can be stored for several months at -20°C without deterioration.

Tissue Preparation:

Fixation:

Tissues are fixed in the conventional way with concentrations of 2 to 5% Glutaraldehyde and rinsed with the buffers.

Dehydration:

Dehydrate according to the following schedule with aqueous dilutions of GACH.

20% GACH + 80% H20 3 hours or overnight

50% GACH + 50% H20 2 - 4 hours

80% GACH + 20% H20 2 - 4 hours

100% GACH 1 - 2 hours

This procedure is carried out in a cold room or in vials immersed in ice (1°C).

Particular specimens, (e.g., erythrocites) can be embedded in the undiluted medium, suspended directly in 100% GACH, [Dodge, et al]).

Embedding is carried out by removing the tissue from the last 100% GACH and placing it on a small droplet of fresh GACH on a dental wax plate at room temperature.

Polymerization is obtained by placing the plate in an incubator at 37°C for 8 - 14 hours.

Sectioning:

The droplets of polymerized GACH can be cemented on blank epoxy blocks, trimmed and then sectioned on the ultramicrotome using diamond or glass knives.

Polymerized GACH is no longer soluble in water; therefore, the sections can float on the surface of the water in the boat of the knife. The thickness of the sections can be judged by interference color similar to epoxy sections.

To increase contrast on the cellular components, sections can be stained with aqueous Uranyl Acetate and Lead Citrate.

Reference:

Dodge, J. T.; Mitchel, C. - Arch. Biochem. biophys. 100 - 119 (1963)