

For routine decalcification of tissue in histology, based on buffered Formal-Formic acid

The mineral calcium of bones can be considered as hydroxyapatite, calcium ions are removed by the action of hydrogen ions.

As the calcium is progressively removed by the decalcifier the concentration of calcium ions builds up in the reagent along with a falling hydrogen ion concentration therefore it is necessary to change the decalcifying reagent frequently to remove the calcium ions and restore the hydrogen ion concentrations.

Good fixation is mandatory before decalcification for good results it will also prevent the swelling and displacement of the undenatured collagen and other protein components of the bone.

Excess skin should be removed and large specimens sawn into slices.

During decalcification debris can occur and this should be washed away under a stream of deionised water to prevent it being embedded in the tissue.

Decalcification time is quick (depending on sample) several hours up to 2 days.

It is important to determine the end point of decalcification so that tissues are not destroyed and the ability of basophilic chromatin to stain is not depleted.

A general procedure is as follows:

1 – Try to keep specimen thickness to 3mm.

2 – Place it on a bed of non-adherent lint in a 250ml flask / beaker.

3 – Cover specimen with 250ml decalcifier and cover top with foil, or plastic film. Maintain temperature at 18-25 Deg C. Higher temperatures in a microwave oven can be used (max 55 Deg C) this will produce around a 10 fold increase in the rate at which decalcification takes place, however there is a greater risk that of tissue damage at these temperatures.

4 – Change the decalcifier 2-3 times per day and test daily for end-point determination.

Determination of end-point:

If the specimen is large enough, extends beyond the region of interest and contains calcified tissue throughout, it may be trimmed with a scalpel regularly during decalcification. When it can be cut easily, it will also be soft enough to be sectioned by a microtome.

A specimen of unwanted bone of approximately the same size as the sample can be processed alongside the specimen to monitor the decalcification. When this unwanted specimen is fully softened, the specimen for study should also be decalcified.

Do not test specimens by poking/pressing needles into them.

Chemical Test to determine end-point decalcification.

A more complete test for determining decalcification is as follows, based on the property that calcium oxalate is insoluble in neutral water or aqueous alkali.

1 – Add drops of SG0.880 (approx 30-35%) Ammonia solution to 5ml of used decalcifier until the mixture is alkaline to Litmus pH 7.5-9.0

2 – Add 5ml of 3% w/v ammonium oxalate (aq) and leave to stand for 30 minutes.

Result: If no white precipitate has formed after this time, the reagent contains no calcium ions and decalcification is complete.

Material Safety Data Section

This product contains a mixture of Formic acid and Formaldehyde < 20% in aqueous solution.

This product is CORROSIVE to all body tissue and HARMFUL by ingestion and inhalation and skin contact. Wear suitable protective when using. Do not consume. Keep stored tightly closed in a safe place.

Eyes – If in contact wash with water or apply eye bath for 5 minutes. If soreness persists seek medical treatment.

Skin – If in contact wash with plenty of water then soap & water.

Ingestion – Wash out mouth with water, do not allow rinse to be swallowed. It may be wise to drink a small amount of water to rinse down and dilute the product then seek urgent medical advice on what treatment to administer. Show this leaflet to medical professional.

Inhalation – Move patient to clear air zone apply oxygen if required and seek urgent medical attention on what treatment to administer.

Waste disposal.

Dilute product to public sewer with large amounts of water or neutralise product with sodium carbonate and dilute to public sewer. Consult local regulations before disposal.

Unsatisfactory performance

As part of our duty to monitor product performance and our policy of continual improvement. Please report to us any unsatisfactory performance you may experience with this product. If any reagent degrades before expiry of shelf life we will replace that reagent free of charge. GCC Diagnostics guarantees the quality of this product, the user should however determine the suitability of this product for their intended use.

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