

For routine decalcification of tissue in histology, based on EDTA.

The mineral calcium of bones can be considered as hydroxyapatite, chelating agents such as EDTA are organic compounds which are able to bind (chelate) certain metals. EDTA is able to bind calcium, therefore it finds use as a decalcification agent. It is a slow process as calcium is removed layer by layer from the hydroxyapatite lattice. Tissues decalcified by this method show a minimum of artifact and may subsequently be stained by most techniques with first class results. These qualities have made EDTA the decalcification agent of choice in electron microscopy.

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 slow but gentle taking (depending on sample) several days or weeks.

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 up to 60 Deg
- 4 – Change the decalcifier once 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 needles into them.

Chemical test to determine end point.

A more complete test for determining decalcification is as follows, based on the property that calcium EDTA 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.

Safety Data Section

This product is based on water soluble EDTA salts and as such poses little risk to Health & Safety in normal use. Wear suitable eye protection and follow the usual good laboratory practice in handling laboratory reagents.

If irritation to eyes or skin occurs rinse well with water and seek medical attention if soreness persists. If ingested seek medical attention if you feel unwell.

Waste disposal.

Dilute product to public sewer with large amounts of water . 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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