

ANIMAL TISSUE TECHNIQUES

Fourth Edition Gretchen L. Humason

Decalcification

Calcium deposits may be so heavily concentrated in the tissue that they may interfere with sectioning and result in torn sections and nicks on the knife-edge. If deposits are sparse, overnight soaking of blocked tissue in water will soften the deposits sufficiently for sections. Heavy deposits may be removed by any of several methods, but do not leave tissue in any of the fluids longer than necessary.

If any doubt arises about the completion of decalcification, check for calcium by the following method:

To 5ml of the solution containing the tissue, add 1ml of 5% sodium or ammonium oxalate. Allow standing for 5 minutes. If precipitate forms, decalcification is not complete. A clear solution indicates it is complete. Sticking needles in the tissue to check hardness is a sloppy technique that can damage cells.

An excellent decalcifying fluid, **RDO**, can be purchased by the gallon. After using **RDO** for several years, I recommend it as superior to other solutions. Its rapidity of action is remarkable and quality of staining and histological detail following its use is excellent. Old bones cut down to 1 cm in thickness, if possible, require a 6-hour treatment; small and young pieces only 1-2 hours. Teeth will require overnight and up to 18-24 hours. Do not over decalcify; this detracts from the staining quality. Decalcifying may be followed by brief washing in water, but this is not necessary. Fixation and decalcification may be combined in a mixture of 1 part undiluted formalin with 9 parts **RDO**.

Manufacture's Note

The combination of **RDO** and formalin is discouraged, but should always be done under a fume hood to ensure the removal of potentially harmful vapors. Always follow the suggested directions for use. Please contact **APEX ENGINEERING PRODUCTS** with any questions you may have about the use of **RDO**.