

## Product Information

### **DeltaCAL – Decalcifying reagent**

#### **Use:**

For in vitro diagnostic use. **DeltaCAL** is designed to be a very RAPID and effective decalcifying agent. It is intended to be used for decalcification of routine, bone marrow core and immunohistochemical specimens. **DeltaCAL** can be tailored to suit your specific lab routine and to the individual type of specimen requiring decalcification.

#### **How It Works:**

The calcium found in bone (teeth, etc.) is mainly carbonate and phosphate salts which are only slightly soluble in water. **DeltaCAL** acts to release calcium from its combination with these anions and affects an ion exchange to give a soluble calcium salt. The calcium ions are effectively removed from the specimen and remain in the decalcifying solution.

**DeltaCAL** is unique in its ability to work very rapidly. Careful monitoring should be employed to avoid over decalcification which will lead to the potential loss of basophilic properties.

**DeltaCAL** will provide excellent nuclear staining, and allows superior H & E and immunohistochemical staining results.

#### **Directions for Optimal Decalcifying:**

**1. DeltaCAL** is corrosive on metal so all decalcifying must be performed in plastic or glass containers. The solution has a pale brown color and a dark brown sediment may appear on standing. The sediment does not change the effectiveness of the solution, but it can be filtered out without loss of effectiveness.

Do not over decalcify. The action of **DeltaCAL** is very rapid, in most cases it occurs in 4 hours or less. Overnight decalcification should be avoided (exception – teeth and extremely dense bone can be decalcified overnight if monitored carefully). If specimen decalcification is incomplete at the end of the work day, remove from **DeltaCAL**, rinse in tap water to remove residual solution and place in 10% NBF until the procedure is resumed.

**2.** For optimum sectioning, staining, and nuclear detail, specimens must be completely fixed prior to decalcification. Avoid the combination of formalin and **DeltaCAL** by washing specimens briefly in tap water prior to decalcification. (**Note:** the liquid combination of formalin and an HCl acid solution will form bis-chloromethyl ether, a potent carcinogen). Proper fixation has proven to be the most important step in the processing of tissue specimens. Due to the introduction of antigen retrieval and other unmasking procedures, longer (adequate) fixation times should not interfere with IHC techniques.

**3.** Most mature bone sections of 1 cm size will decalcify in 4-6 hours; smaller cancellous bone only requires 2-4 hours; bone biopsies will decalcify in 20-60 minutes. Avoid over decalcification on all specimens as it will harden the tissue creating poor cellular detail and difficulty in determining the endpoint. **DeltaCAL** is a *rapid decalcifier*. If a specimen is over

decalcified, the nuclear staining can be improved by longer times in the hematoxylin or by neutralizing the deparaffinized tissue section with lithium carbonate or 4% sodium bicarbonate before staining in hematoxylin. The morphology of the tissue starts to be destroyed as soon as the specimens are completely decalcified and left in the acid solution. This destruction will occur rapidly.

Due to the addition of polymers in paraffins, there is better support to aid in the sectioning of decalcified bone specimens, so as to reduce the need to reach the "ideal" endpoint for a specimen for decalcification. In fact, specimen blocks that fail to section because of incomplete decalcification may be placed in a decal solution for a short period of time, rinsed, and sectioned easily.

4. Use a volume of **DeltaCAL** to tissue in a 20:1 ratio or better. Frequent mild agitation or swirling of the specimen in solution will enhance more even penetration and decrease exposure time in solution. This will also minimize over decalcification of the outer tissue or bone before sufficient core decalcifying is achieved. Small biopsies and bone samples will not require agitation.

5. To avoid over decalcification, check the specimen at regular interval for endpoint\* via whichever method the institution follows (x-ray, flexibility, chemical analysis).

6. When decalcification has been determined to be complete, thoroughly rinse the specimen prior to processing to ensure excellent quality in nuclear detail.

#### **\*A Chemical Test to Determine the Endpoint of Decalcification**

Take 5 ml of **DeltaCAL** from the bottom of the specimen container. To this solution add 5 ml of 5% ammonium oxylate. Add 5 ml of 5% ammonium hydroxide. Let this solution set for 10 minutes. If a precipitate forms in this solution, decalcification is NOT complete and the specimen should remain in the **DeltaCAL**.

*It is not recommended to reuse **DeltaCAL** to achieve optimum decalcification and standardization of procedures. The nature of a decalcifying agent is to release calcium ions from the bone into the acid solution. As the solution becomes saturated with calcium ions, the decalcification process will slow substantially.*

#### **Storage and Disposal**

Some change of color or an increase in precipitate may occur after long periods of storage. **DeltaCAL** may be filtered if desired without altering its effectiveness. **DeltaCAL** is biodegradable as received and may be disposed down regular city sewer systems with a water flush according to federal, state and local regulations. **DeltaCAL** will discolor and corrode most metals. Avoid exposure to metal cassettes, countertops and slide racks. Flush **DeltaCAL** with water to prevent damage to chrome plated plumbing fixtures. Store at room temperature. Keep container closed. **DeltaCAL** has a five-year shelf life. Lot number and expiration date are on the label.

#### **Precautions**

Under normal conditions **DeltaCAL** should not be considered hazardous. As with most acid solutions, it is recommended to avoid extensive or repeated contact. If eye or skin contact occurs, flush affected area with water.

