**RDO IN VITRO DIAGNOSTIC USE**

*RDO* is a very rapid bone decalcifier. The acid component of *RDO* reacts with calcium in mineralized tissues to form soluble calcium salts. The little extra care required in its use will result in routine sections with superb histological detail and staining characteristics in a fraction of the time required with other decalcifiers.

Tissues should be thoroughly fixed before decalcification. Most standard fixatives can be used prior to *RDO* use. To insure adequate fixation and decalcification, specimens should be trimmed to less than 1 cm thickness. FORMALIN FIXATION AND *RDO* DECALCIFICATION SHOULD NOT BE COMBINED. Hydrochloric acid (active ingredient of *RDO*) and formaldehyde vapors have been reported to form a potent carcinogen, bischloromethyl ether. Prior fixation with formalin is permissible. Brief washing in water before *RDO* decalcification is advised. Do not use metallic equipment/cassettes for decalcification. *RDO* corrodes most metals after long periods of exposure. Decalcification tissues may be placed in metallic equipment after washing.

DO NOT OVER DECALCIFY. *RDO* action is very rapid. DO NOT leave bone specimen in *RDO* for several days as required with most commercial decalcifiers. MOST SPECIMENS CAN BE DECALCIFIED IN FOUR HOURS OR LESS. Use adequate volume of *RDO* to tissue; a 20:1 volume ration of *RDO* tissue is recommended. The key determinations for time required for decalcification are size and density of the specimen. Most mature bones of 1 cm size are decalcified in 4-6 hours; smaller cancellous bone in 2-4 hours. Bone biopsies are decalcified in 30-60 minutes. Teeth and entire femur heads may require overnight treatment. If *RDO* action is too rapid, dilute with distilled or deionized water. Good results have been obtained on bone marrow biopsies with a 3:1 dilution (*RDO* to water). Determine the point of decalcification using standard methods (ie. X-ray, flexibility, chemical analysis).

Proceed with routine processing and embedding. Washing tissue prior to processing is optional.

Overexposure to *RDO* can result in poor hematoxylin staining. If this occurs, satisfactory results can be obtained by treating deparafinizing slides to hematoxylin with aqueous saturated lithium carbonate (1-2 minutes) or 10% aqueous sodium bicarbonate (6-8 hours). Poor histological detail/artifacts (swelling, fragmentation) can also occur from excess decalcification. Hemosiderin is not removed by *RDO*.

**STORAGE AND DISPOSAL**

Store at room temperature. Keep container closed at all times. Store only in a glass or plastic container. Do not use metal containers as *RDO* will irreversibly corrode aluminum, nickel, and some stainless steel equipment.
After long periods of storage some change of color or an increase of suspended precipitate may occur. These are normal occurrences and do not affect the decalcifying potential of RDO. The precipitate may be allowed to settle or removed by filtration; however, neither action is necessary.

RDO is biodegradable as received and may be disposed of down regular city sewer systems with water flush. Dispose of according to federal, state, and local regulations. Care should be taken to insure the finish of chrome plated plumbing fixtures.