




RDB Rapid decalcifier modified solution technical information  
Security card 02-106  
Product code 02-106  
Pack 1000 ml or on request  
Stability of product properly conserved at 4°C 24 months

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in case of emergency UE number		112
in case of emergency UK number		999
en cas d'urgence Suisse number		145

## Description

RDB is designed to be a universal effective decalcifying agent. It is intended for the decalcification of routine, immunohistochemical and bone marrow core specimens. RDB has been tailored to suit your specific lab routine. It works equally well with all types of specimens in an easy-to-handle time frame.

RDB is an aqueous solution of nitric acid and proprietary compounds.

Precautions and disclaimer. This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

## Ready to use solution.

Some change of color or an increase in precipitate may occur after long periods of storage. RDB may be filtered if desired without altering its effectiveness

## Principle

Decalcification methods are necessary for optical microscopic examinations of bone and other hard tissue in routine histological procedures. The material to be decalcified is placed in an excess of decalcifying solution and demineralised (decalcified) in this. The decalcification time is dependent on the size and structural density of the hard tissue, while the composition of the decalcifying solution also exerts a decisive influence on the process.

Decalcification of bone and hard tissue requires the use of either inorganic acid, as is the case with RDB, which liberate the acids of the mineral salts and can subsequently be rinsed out.

The decalcifying solution has been modified to facilitate decalcification, the solution is a black dark colour. The ingredient used don't stain the tissue to be decalcified.

## Application

Decalcification and fixation of bone, hard tissue and keratinized tissue. Specimens must be completely fixed prior to decalcification. Fixation has proven to be the most important step in the processing of tissue specimens. With the introduction of various unmasking procedures, longer fixation times should not interfere with immunohistochemical techniques.

## Note

Do not use RDB for sensitive tissue, e.g. punched iliac crest specimens.

## Sample material

Bone and hard tissue (teeth) and keratinized tissue (acuminate warts, nails) for use in paraffin sections in histology.

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### Sample preparation

Place all the tissue to be decalcified into a vessel (glass or plastic) containing an excess of the ready-to-use RDB decalcifying solution and decalcify completely in this solution.

### Procedure (please note decalcifying must be done at 4°C)

RDB acts faster than most decal solutions. Use a 20:1 ration (v/v), or higher. In most cases decalcification occurs in 6 hours or less. Overnight decalcifying 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 day, remove the tissue from RDB, rinse in tap water to remove residual solution, and place in 10% neutral buffered formalin until the decalcification procedure is to be resumed.

Wash the tissue free of the formalin solution before the tissue is placed back in RDB.

When placing the specimen back into RDB, frequent or mild agitation or swirling of the specimen in solution will augment even penetration and decrease the exposure time needed to complete the procedure.

Most mature bone sections of 1 cm size will decalcify in 6-8 hours. Smaller cancellous bone only requires 4-6 hours. Bone biopsies will decalcify in 20-60 minutes. Avoid over-decalcification on all specimens, as it will harden the tissue and create poor cellular detail and difficulty in determining the endpoint.

If the decalcification is incomplete, the paraffin block may be placed in RDB for a quick surface decalcification. Time in RDB should not exceed 12-36 hours for most 1 cm mature bone sections, 6-18 hours for smaller cancellous bone or 2-15 hours for bone marrow biopsies. Overnight decalcification is recommended for mature bone, teeth and entire femur heads.

Frequent mild agitation or swirling of the specimen in solution will enhance even penetration and decrease the exposure time of the tissue to the acid solution. This will also minimize over decalcification of the outer tissue or bone before sufficient core decalcifying is achieved. To avoid over-decalcification, check the specimen at regular intervals for endpoint. Check every 2 hours for mildly calcified specimens and every 12 hours for compact bone.

If a specimen is over decalcified, the nuclear staining can be improved by longer times in the haematoxylin or by neutralizing the deparaffinised tissue section with a saturated solution of lithium carbonate or a 4% sodium bicarbonate solution before staining in haematoxylin. The morphology of the tissue starts to be destroyed as soon as the specimens are completely decalcified and left in the acid solution.

Staining are carried out according to the standard procedures.

Determining the endpoint of decalcification can be determined with the following chemical test. Check specimen every 2 hours for mildly calcified specimens and every 12 hours for compact bone.

1. Take 5 ml of RDB from the bottom of the decal container.
2. To this, add 5 ml of 5% ammonium oxalate
3. Add 5 ml of 5% ammonium hydroxide
4. Let the solution set for 15 minutes.
5. If precipitate forms, calcium (calcium oxalate) is present and decalcification is not complete

### Notes on use

Too long a decalcification of the tissue can result in the destruction of the morphological structure of the specimen and thus negatively affect the subsequent nucleus staining.

Immunohistological methods cannot be employed after decalcification with RDB, since the antigen structures of the tissue can no longer be detected.

If immunological procedures are required for the diagnosis, then RDO, 02-102-103, an antigen-maintaining decalcifying solution on an EDTA base, must be used.

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#### Diagnostics

Diagnoses must only be made by authorized and trained persons.

Valid nomenclatures must be used.

Further tests must be selected and performed according to recognized methods.

#### Storage

Store the solution at 4°C. After first opening, the bottle can be used up to the expiry date when stored at 4°C.

#### Shelf-life

The solution must be used by the expiry date stated.

The bottles must be kept tightly closed at all times.

#### Instructions for use

For professional use only.

The specimens must be taken by authorized and qualified personnel. The specimens must be taken using state-of-the-art technology. In order to avoid errors, the decalcification process must be carried out by qualified personnel. National guidelines for work safety and quality assurance must be followed. Laboratory-internal SOPs for the reagents used must be followed.

#### Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Attention must be paid to the safety data sheet.

#### Instructions for disposal.

Used solutions and solutions that are past their shelf-life must be disposed of as special waste according to local disposal guidelines.

Please observe the hazard classification on the label and the information given in the safety data sheet.

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