DDK Italia

IVD dispositivo medico-diagnostico in vitro CE

RDL Decalcifier with fixative slow actions technical information
Security card 02-105
Product code 02-105
Pack 1000 ml or on request
Stability of product properly conserved at 15-20°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
Bone decalcifying solution for bone and hard tissue in histology

Principle
Decalcification methods are necessary for optical microscopic examinations of 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 respective tissue, while the composition of the decalcifying solution also exerts a decisive influence on the process. When decalcifying sensitive, calcium-containing tissue a solution such as RDL is used, which contains complex- or chelate-forming agents that bind the calcium ions of the tissue.
This type of decalcifying solution preserves the antigen structures in the tissue, with the result that immunological procedures can be conducted.

RDL 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.

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. RDL 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 RDL, which liberate the acids of the mineral salts and can subsequently be rinsed out.

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.

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Sample material

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

Sample preparation

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

Note

Sample material to be decalcified with RDL do not need to be fixed previously.
Place all the tissue to be decalcified into a vessel (glass or plastic) containing an excess of the ready-to-use RDL decalcifying solution and decalcify completely in this solution.

Procedure

Iliac crest and other hard tissue. The decalcification time and the quantity of RDM required are dependent on the size, type, and density of the respective tissue.
A piece of bone 15 x 9 x 3 mm in size, taken from e.g. the iliac crest, is immersed in approx. 50-100 ml of RDL and has a decalcification time of 18-24 hours.

Compact, calcified tissue

Compact, calcified tissue 1.5 x 1 x 0.3 cm in size is decalcified after 48- 72 hours.
If no Immunohistological procedures are planned, decalcification can be carried out more rapidly with RDB 02-106.
Determining the end-point of the decalcification process In the case of e.g. iliac crest tissue, the end of the decalcification process is reached when the tissue floats in the solution.

Result

Decalcified tissue is cartilaginous or rubberlike in its consistency and exhibits only a weak resistance.
The decalcified tissue is processed further for histology in the same way as other materials of the corresponding size.

If the decalcification is incomplete, the paraffin block may be placed in RDL for a quick surface decalcification. Time in RDL 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 is 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 RDL 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

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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 RDL, since the antigen structures of the tissue can no longer be detected.
If immunological procedures are required for the diagnosis, then 02-102-103, an antigen-maintaining decalcifying solution on an EDTA base, must be used.

Sample preparation
All samples must be treated using state-of-the-art technology. All samples must be clearly labelled.
Suitable instruments must be used for taking samples and for their preparation. Follow the manufacturer's instructions for application/use.

Diagnostics
Diagnoses must only 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 15-20°C.
After first opening, the bottle can be used up to the expiry date when stored at 15-20°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.