

Instructions for use

Decalcifier soft

6484

Histological Decalcifier on base of EDTA

The Decalcifier soft is used for gentle decalcification of bones and other calcified tissues. The solution contains 25 % ethylenediamine tetra acetic acid (EDTA), a weak organic acid.

The main component of bone tissue is hydroxyl apatite. In a watery solution it dissolves at a low percentage, building out a balance between solid apatite and its saturated solution. EDTA has chelating properties. It binds the calcium ions of the solved apatite in chelate complexes. This leads to a chain reaction: To maintain a constant level of calcium in the solution new calcium ions are solved from the tissue and bounded as well in chelate complexes. In the end of the reaction the tissue is completely demineralized and ready for cutting and preparing. This process occurs in a weakly alkaline milieu and is, therefore, much more tissue-conserving than the decalcification with strong acids. Nucleic acids and enzymes are not affected. Subsequently, *in situ*-hybridizations, enzymohistochemical and immunological assays can be carried out.

The process of decalcification takes more time than acid decalcification. Therefore, for routine work we recommend our Decalcifier standard (6483) on base of trichloroacetic acid.

Application

The decalcification takes place at room temperature. Put the fixed tissue into the decalcifier solution (ratio tissue to decalcifier 1:20) and make sure that the tissue is completely covered with liquid.

The length of the decalcification depends on the size and type of the material. Bone biopsies need ca. 3-4 days until complete decalcification. The procedure can be accelerated by using a magnetic stirrer. Change the solution at least once every 24 hours.

The end-point of the decalcification can be determined by puncturing the material at an area of less relevance for the diagnostic procedure. The tissue must be completely rubber-like. Another possibility is an ammonium oxalate test to find out if there are still calcium ions in the solution: Remove a small amount of the used decalcifier solution and adjust the pH value with ammonia solution to > 7. Then add the same amount of ammonium oxalate solution 3%. After 30 min there must not be any turbidity (calcium oxalate). Otherwise, the decalcification procedure must be continued. Avoid a too long residence time of the solution.

After the decalcification it is necessary to rinse the tissue well in flowing tap water to remove the residues of EDTA, which would precipitate when coming in contact with alcohol. Only then dehydrate with alcohol. After a clearing step with clearing agent embed in paraffin.

Storage

The solution should be stored tightly sealed at room temperature. Shelf life is minimum 2 years after manufacture.



Danger H290-H314-H373 P280-P302+P352-P305+P351+P338-P310

Full text of hazard- and precautionary statements
see material safety data sheet section 2.2

Decalcifier soft

500 ml	glass	6484.1
1 L	glass	6484.2
2.5 L	plastic	6484.3
5 L	plastic	6484.4

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sse 06/2021

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