LR White for Hard Tissue

EMS Catalog #14380-14382

LR White can be used for the microtomy of decalcified bone and teeth and also for microtomy or "sawing or grinding" of undecalcified tissues.

Decalcified Tissue

Decalcified tissue may be processed, cut and stained similarly to soft tissue (see using LR White for Light Microscopy), except that dehydration and infiltration times may need to be extended depending on the size of tissue. It is also recommended that bone be "de-fatted" to improve the penetration of resin into marrow cavities. This can be achieved by using chloroform after dehydration, returning to absolute alcohol to remove the chloroform before infiltrating with resin and polymerizing.

Undecalcified Tissue

Dehydration and infiltration times will vary depending on size and density of tissue. Those laboratories using Methyl or Butyl Methacrylate at present can use similar dehydration times, but infiltration will probably be shortened due to the low viscosity of the resin.

Dehydration

A graded series of alcohols should be used for dehydration of tissue, and when processing bone "de-fatting' is recommended to improve the penetration of resin into marrow cavities. This can be done using chloroform, for the same length of time that would be necessary to clear the tissue. The bone should then be taken back to absolute alcohol and given sufficient changes to remove the chloroform before infiltration with LR White (Hard grade).

Infiltration

Several changes of resin will be necessary and impregnation under vacuum is recommended.

Polymerization

The tissue can be heat or accelerator cured after embedding in strong plastic molds, such as JB4 or Peel-A-Way type, or aluminum foil dishes. (EMS #70176-10 to 70176-30).

When heat curing the molds should first be filled with resin then the tissue added and orientated. Polymerization will occur in 18-24 hours at 60° C ± 2° C. The surface of the block exposed to oxygen may remain slightly sticky, but this will not affect the cutting quality of the face of the block. Some ovens are not capable of controlling temperature so closely and if faced with over brittle blocks this is the first parameter to check.

When accelerator or cold curing the molds should be placed in a bath of ice cold water to disperse the heat produced during the exothermic polymerization. The base of the molds should first be smeared with accelerator using a cotton wool bud or swab, the accelerator is then added to the resin, 1 drop per 10 ml resin, and thoroughly mixed before pouring into the mold, the tissue is then placed into the mold and orientated. Polymerization should occur in 10-20 minutes, if it occurs faster than this we recommend either more careful metering of the one-drop of accelerator or a higher volume of resin per drop of accelerator. NB: the accelerator does have some toxic risk and contact with skin and eyes should be advised.

Cutting and Mounting

Bone marrow trephines and small pieces of cancellous bone may be cut using a motorized heavy duty microtome, but larger pieces of cancellous bone, cortical bone and teeth offer too much resistance to the microtome knife and preparations of this material must be prepared by sawing and grinding.

Microtomy

Sections can be cut, using Ralph type glass knives for trephines or a tungsten carbide knife for larger pieces of cancellous bone, from 2–10 micron. Blocks can be cut dry, the sections picked up and floated out on a hot plate at 60° – 70° C using the following solutions: to 20 ml acetone add 0.5ml benzyl alcohol mix then make up to 50ml with distilled water. A section adhesive such as egg albumin can be added to this if required. Sections should be allowed to dry on the hot plate for at least 30 minutes before staining.

Sawing and Grinding

Thick slices 150–200 micron can be cut using a milling machine and then ground to the required thickness, usually 20 micron for staining of 70 micron for microradiography; the section is inclined to fragment if grinding is continued much below 20 micron.

Using the newer types of saw microtome, such as the Leitz 1600, which has a diamond coated internal hole saw, sections can actually be cut at 20 micron and no further grinding is necessary.

Section Staining

Sections of material embedding in LR White are stained "free floating", times of staining are usually longer than those for paraffin, sections and dehydration through alcohols should be avoided. A recommended schedule for Hematoxylin and Eosin staining is as follows:

1. Remove Ca deposits, which would otherwise interfere with the staining, from the section by treating it with Kristensen's Decalcifying Solution for about 15 minutes.
2. Wash in running tap water for a few minutes to remove the formic acid from the tissue.

3. Transfer the section to several changes of distilled water, a few minutes each and then into a 0.5% w/v solution of periodic acid in distilled water where it is left for 5 minutes.
4. Wash the section with several changes of distilled water and stain with Harris Hematoxylin for about 1 hour.
5. Transfer the section, after a short time in distilled water, into running tap water to “blue” the Hematoxylin stained tissue.
6. Check the Hematoxylin stain; if the tissue should be overstained or the surface of the resin has become stained with Hematoxylin, this may be corrected by a short differentiation in acid alcohol (0.5% HCl in 70% ethanol) and “reblueing” of the stained tissue.
7. Rinse the section in distilled water and Counterstain it, using a 5% solution of Eosin Y in distilled water; leave the section in the Eosin stain for 30 minutes to 1 hour, wash briefly in running tap water and check the staining of the tissue. Nuclei and Hematoxophilic elements should be bright blue, cytoplastic structures in various shades of red–pink.
8. Rinse the section in distilled water, blot dry with filter paper and either clear briefly in Xylene and mount in DPX, or mount directly in LR White resin by adding a drop of accelerator to 1 ml of resin.