

## *L.R. White Resin*

*Catalog Number 17411-500*

## *Accelerator for L.R. White (Room Temperature or Cold Curing)*

*Catalog Number 17413-10*

### **Introduction:**

L.R. White is a low viscosity acrylic embedding medium for routine use in light and electron microscopy, histochemistry, and immunohistochemistry. Some acrylic resins do not stand up well in the electron beam and may degrade quickly. Epoxy resins cannot be used for light microscopy due to poor staining with routine protocols. L.R. White provides an alternative to these plastics providing the advantage of performing both light and electron microscopy often with one block.

Light microscopy embedments of L.R. White provide better resolution with improved cellular definition when compared to either paraffin or frozen sections. Most routine histological stains penetrate very well with some time adjustments. Larger blocks can be trimmed down for electron microscopy allowing thin sectioning and appropriate staining. The properties of L.R. White make it useful in the diagnosis of renal disease, lymphomas, and bone marrow trephines. Its ability to be used for both light and electron microscopy makes it a very useful resin for research.

L.R. White can be used for the histochemical demonstration of some resistant enzymes and for intracellular immunoglobulins in immunohistochemistry. The number of antibodies with good to excellent staining has not been determined.

### **Fixation:**

To prepare combination blocks for both Light (LM) and Electron (EM) Microscopy, use Poly/LEM Fixative (Cat. # 16864). Poly/LEM Fixative is 10% Neutral Buffered Formalin with no methanol added.

EM fixation with freshly prepared 3 - 4% paraformaldehyde or EM Grade Methanol Free Formaldehyde in PBS at pH 7.2 with 2.5% sucrose maintains the cellular integrity and staining intensity for both types of microscopy.

Glutaraldehyde - formaldehyde mixtures often cause light or patchy staining with hematoxylin and eosin stains for LM. Standard buffered formalin fixation is often poor for EM structural studies.

Avoid osmium tetroxide fixation for combination blocks as it will interfere or prevent routine LM staining. Adding 1% phosphotungstic acid (w/v) (Cat. # 23224) to the first absolute ethanol dehydration step will improve electron density under the beam while not interfering with LM staining. If this does not give enough contrast for EM,

postfix and stain the ultra-thin sections with 1% osmium tetroxide followed by a brief rinse. Exposing sections to osmium tetroxide vapor on a copper grid under a hood will also postfix the sections. Always use osmium tetroxide under a hood, with gloves and appropriate protection from fumes. Lead acetate staining will also increase contrast for viewing.

### **Dehydration:**

Treat specimens with standard dehydration times in graded alcohols. Do not use Acetone with L.R. White. It acts as a free radical scavenger and inhibits the polymerization process. Dehydration times for a block prepared for LM (12mm length x 10mm width x 3mm thickness) is:

1. 70% Ethanol	2 changes	30 minutes each
2. 95% Ethanol	2 changes	30 minutes each
3. Absolute Ethanol	2 changes	30 minutes each

**Infiltration:**

L.R. White has an extremely low viscosity, enabling shorter infiltration times in the resin. Perform infiltration with resin only. Infiltration times will depend on the tissue size and density.

1. L.R. White Resin (Only) 2 changes 1 hour each (Minimum)
2. L.R. White Resin (Only) 1 change 1 hour or overnight

Use a gentle rocker table or wheel to assure proper infiltration for all tissues.

**Embedding:****Room Temperature Curing**

Room temperature curing may give slightly better cutting properties for LM while thermal curing is better for EM examination.

The embedding solution is made by adding 20 $\mu$ l (*with a calibrated micropipette and tip\**) of L.R. White Accelerator to 10ml of L.R. White resin just prior to use and mixing well.

**\* Using a calibrated micropipette and tip is the only accurate way to measure 20 $\mu$ l. Using a disposable or non-calibrated pipette to estimate one drop or 20 $\mu$ l can result in the addition of too much accelerator. This will cause the exothermic reaction to occur too quickly resulting in bubbling and over-heating of the specimen. Exact measurement of 20 $\mu$ l will produce consistent blocks every time.**

L.R. White Resin with added accelerator should polymerize in 10 to 20 minutes at room temperature. The addition of too much accelerator will cause the resin to polymerize faster. It is not necessary to exclude air for room temperature polymerization. The exothermic reaction during polymerization causes the generation of heat. The blocks should be cooled to room temperature prior to removal from the molds.

To reduce the exothermic reaction, L.R. White can be polymerized at 0°C to 4°C. Capping the BEEM® capsules and using block holders is advised for cold polymerization to prevent contact with water and to assist with the polymerization process.

For larger specimens, place the molds in an ice bath or quickly fill and move to a 4°C refrigerator for several hours to overnight. Cold polymerization occurs in 45 minutes to 1 hour for BEEM® capsules on ice. Larger molds may require several hours for cold polymerization. In this case,

refrigeration is the best method. The polymerization will layer from the bottom up. Do not check the progress of the polymerization by lifting the capsule or block holder.

**Thermal Curing**

Thermal curing with L.R. White requires no mixing of components. Use L.R. White Resin directly from the bottle to fill JB-4 molds or BEEM® capsules either before or after the tissue has been oriented in the correct plane. The BEEM® capsules must be capped and the JB-4 molds must have block holders in place to exclude oxygen, which will prevent complete polymerization. Place filled molds in a 60°C to 65°C oven for 12 to 20 hours for polymerization. Cool the blocks to room temperature prior to removal from the molds.

**Storage of LR White Resin and Accelerator:**

L.R. White should be kept at 4°C. Only the amount required should be removed from the bottle and allowed to come to room temperature. The cap should be replaced immediately and kept tight at all times to reduce oxygen in the solution and the absorption of water from humidity and condensation. The addition of oxygen or water can inhibit polymerization during embedding. The accelerator can be stored in a cool dark area at room temperature.

**Sectioning and Mounting**

L.R. White will require a microtome that is designed to section plastics. Routine paraffin microtomes are not appropriate for plastics, as they cause chatter in the sections. Replace glass knives with tungsten carbide triangular knives for small blocks. Larger blocks require either the larger tungsten carbide blades or glass Ralph knives. Section smaller block faces as thin as 0.25 $\mu$  with either knife type. Section larger blocks at 1.0 $\mu$  to 5 $\mu$  with tungsten carbide blades. Thicker sections may be more difficult to produce due to chatter or shattering of the material.

Use a drop of water to help expand the plastic for mounting on glass slides. An applicator stick soaked in acetone gently waved over the section and water will further expand the section. Place the slide on a hot plate at 60°C to 70°C to evaporate the water and adhere the section to the slide. Use a solution of 30% to 40% acetone and water to float the sections followed by air drying prior to placement on the hot plate. **DO NOT USE ACETONE ON THE HOT PLATE.**

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**Staining**

LM sections of 2 $\mu$  and thicker will stain with most routine stains. Times may need extending to allow penetration of the stains. Solutions made in ethanol or methanol may soften the surrounding L.R. White in the section, lifting it from the slide. Use the shortest times possible with alcohol based stains or substitute with an aqueous base stain where possible. Avoid dehydration through alcohols or shorten to very quick dips. Blot and/or air dry sections to prevent them from falling off the slide.

Use Multiple Stain Solution (Cat. # 08824), an excellent locational stain for very thin sections, to assess the block face or when Hematoxylin and Eosin Y are just too harsh for the sections causing them to float off the slide.

Multiple Stain Solution is a quick stain that is used by either flooding the slide for 15 to 30 seconds or submerging and staining in a 2% to 3% aqueous solution for 1 to 3 minutes followed by a rinse to remove the excess stain. Allow slides to air dry and coverslip to view.

Electron microscopy staining with lead citrate, uranyl acetate, and other heavy metals can be used as routine protocols.

**Safety and Handling Precautions:**

Causes eye and skin irritation. L.R. White should be used under a hood. Gloves should be worn when handling the components for measuring and dispensing.

**Ordering Information:**

Catalog #	Description	Size
17411-500	L.R. White Resin	500g
17413-10	L.R. White Accelerator	100ml

**Additional Products:**

16864	Poly/LEM Fixative
00380	Paraformaldehyde, EM Grade
01921	Acetone, EM grade, 99.5%
09860	Reagent Grade Alcohol (100%)
08824	Multiple Stain Solution
08381	Poly-Mount Coverslipping Media
00224	BEEM® Capsules Size 00
00294	BEEM® Capsules Size 00 Conical
00336	BEEM® Capsules Size 3
00295	BEEM® Capsules Size 00 Bottle Neck
00225	Gelatin, Embedding capsules Size 00 (23.3mm L x 8.18mm W x 0.95mL volume)
07347	Gelatin, Embedding capsules Size 1 (19.0mm L x 6.63mm W x .50mL volume)
07348	Gelatin, Embedding capsules Size 3 (13.9mm L x 5.05mm W 0.21mL volume)

23257	BEEM® Transparent Flat Embedding Mold
23256	Chien 8 Faced Embedding Mold
19440	Chien Universal Embedding Mold
16643A	Polyethylene Molding Cup Trays 6 x 12 x 5mm (20 Cavities)
16643B	Polyethylene Molding Cup Trays 12 x 16 x 5mm (20 Cavities)
17177A	Polyethylene Molding Cup Trays 6 x 8 x 5mm (9 Cavities) Hexagonal
17177B	Polyethylene Molding Cup Trays 2 x 15 x 5mm (9 Cavities)
17177C	Polyethylene Molding Cup Trays 13 x 19 x 5mm (9 Cavities)
15899	JB-4 Block Holders for Molding Cup Trays
24216	Tissue Tack Silane Charged Microscope Slides
22247	Poly-L-Lysine Coated Microscope Slides

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