LR White Embedding Medium

Product Nos. 18181 & 18182

Prod. No. 18181	Medium Grade	500ml
Prod. No. 18182	Hard Grade	500ml

Also Available: Prod. No. 18185 LR White Cold Cure (room temperature) accelerator, 10 ml (use at 1 drop/10ml or 1.5u1/ml of resin), used for chemical accelerator cure.

LR White is a polyhydroxy-aromatic acrylic resin with low toxicity and ultra-low viscosity (8 cP). It can be used for routine electron microscopy or the immunocytochemical localization of antigens by either light or electron microscopy. The polymerized resin is hydrophilic (sections freely permeable to aqueous solutions, even at neutral pH) and has a limited miscibility with water (12% by volume). LR White can be cured by one of four methods: Microwave, heat, UV light (365nm) and chemical (aromatic tertiary amine) accelerators (#18185). For more details of accelerated curing by microwave, see the microwave protocol on page 3.

Please read the following points carefully before using your LR White Resin.

1. **Mixing**: The LR white you have received is supplied with the catalyst, benzoyl peroxide, in a separate vial (9.9g). The benzoyl peroxide **MUST** be added to the LR White Resin prior to its use. The resin will not polymerize without the addition of the catalyst. To mix, pour the contents of the vial into the resin and stir for 30 minutes with a magnetic stirrer. Let the bottle sit at room temperature for another 30 minutes before use. If possible – after addition of the catalyst – let the resin sit 24 hours in the refrigerator prior to use. We have used the resin as quickly as one hour after the addition of the catalyst with successful results. To guarantee that the catalyst is completely dissolved in the resin, the 24 hour time frame is recommended.

2. **Storage/Shelf Life**: After the addition of the catalyst the resin should be stored at 4°C or frozen at up to -80°C for long term storage. The shelf life of the resin begins with the addition of the catalyst. In our experience the shelf life is a minimum of one year when stored at 4°C. If the resin is frozen, allow it to thaw and reach room temperature prior to use.

3. **LR White Protocols**: After the catalyst has been added to the resin it is ready for use in all applications. LR White can act as a solvent. Extended infiltration times in the resin can result in reduced section contrast on the electron microscope. Two processing protocols are outlined. Protocol 1 is normal bench processing for either EM or LM. Protocol 2 is a microwave protocol (Giberson et al. 1997. J. Vet. Diagn. Invest. 9:61-67) which reduces processing times to under 2 hours and minimizes extraction during processing. The microwave protocol was developed using the PELCO® Model 3450 Microwave Processor.

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Protocol 1 – Curing Using Heat, Chemical Accelerator or UV

1. Aldehyde Fixation:
Routine: 1-4 hours in buffered fixative.

Immunocytochemistry: 0.5-2 hours in buffered fixative.
(See Griffiths, G. 1993. Fine Structure Immunocytochemistry, Springer-Verlag, New York, pp 1-89 [Product No. 24918])

2. Buffer Rinse: 2 x 30 min. for both routine and immunocytochemistry.

3. Post Fixation:
Routine: 1 hour in aqueous or buffered 1-2% osmium tetroxide.

Immunocytochemistry: None or reduced osmium (mix equal volumes of 3.0% potassium ferricyanide & 2% osmium tetroxide, final concentrations 1.5:1 – buffered or unbuffered – mix immediately before use). For improved contrast without using OsO_4 see Berryman & Rodewald, 1990. J. Histochem. Cytochem. 38:159 - 70; Berryman et al., 1992. J. Histochem. Cytochem. 40:845-57.

4. Dehydration/Resin Infiltration:
Routine: ETOH (Ethyl Alcohol)
1 x 50% ETOH - 15 min.
1 x 70% ETOH - 15 min. (See Note 5)
1 x 90% ETOH - 15 min.
1 x 100% ETOH - 15 min.
1:1 100% ETOH:Resin - overnight on a rotator
2 x 100% Resin - 1 hour each on a rotator

Immunocytochemistry: (make from fresh 100% ETOH)
1 x 50% ETOH - 15 min.
2 x 70% ETOH - 30 min. ea. (See Note 5)
2:1 Resin:70% ETOH - 1 hour on a rotator
2 x 100% Resin - 1 hour ea.
100% Resin - overnight on a rotator
100% Resin - 1 hour on a rotator

5. Polymerization:
UV: We recommend the use of 2 ea. 15W UV bulbs with the samples positioned -6" (15cm) from the light source and at temperatures between OºC and -20ºC. The PELCO® UVC2 Cryo Chamber (Product No. 6202) is recommended for precise low temperature UV polymerization of resins. Typical cure time using 30W UV is overnight to 24 hours.

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Chemical Accelerator (Cold Cure): Product No. 18185 Accelerator is added to the resin and mixed completely. Pot life at room temperature is volume dependent (the larger the volume the shorter the pot life). The samples can be polymerized in capped 00 BEEM® capsules (Product No. 130), 00 gelatin capsules (Product No. 130-14) or Polyethylene Molding Trays for LM (Product No. 27304 to Product No. 27315). The polymerization rate can be slowed by placing the embedding capsules on ice or in the refrigerator. **Resin polymerization is exothermic**. Typical cure time is 30 minutes to 4 hours, depending upon conditions.

Heat: If 00 gelatin capsules are used the oven temperature can be set as low as 50°C. The use of 00 BEEM® capsules requires that the oven temperature be set to ≥65°C. The caps are removed from the capsules and a square piece of Parafilm® M, greater than the diameter of the cap, is gently pressed into the cap. The capsule is filled with resin and the cap with Parafilm® M is placed on the top to seal. For flat embedding molds made of PTFE are available (Product No. 10506; Product No. 10508). Typical cure time is overnight to 48 hours, depending on conditions (temperatures used).

Protocol 2 – Curing Using Microwave

When employing microwave-assisted tissue processing, routine processing methods are identical to immunocytochemistry. The type of fixative and whether or not to employ post fixation are the major considerations for immunocytochemical applications. The following is our published protocol (Giberson, et al. 1997. J. Vet. Diagn. Invest. 9:61-67) for routine microwave processing done with the PELCO® Model 3450 Microwave Processing System, but applicable to any PELCO® Microwave with a load cooler and variable power.

1. Aldehyde Fixation:
Specimens (1 to 1.5mm^3) are processed in 1.7ml microcentrifuge tubes containing 600µl of fixative. The tubes are microwave irradiated for 40 sec. in a cold spot. The temperature change of the fixative, after irradiation, should be 10-15°C. Let the tissue sit in fixative for 5 minutes, cool the fixative to ≤20°C and irradiate the specimens again for 40 sec.

2. Buffer Rinse:
2 x 2.5 min. outside the microwave.

3. Post Fixation:
The same steps used in the aldehyde fixation step are repeated for the postfixation. Any of the standard post fixatives can be used.

4. Water Rinse:
This is done in the microcentrifuge tubes, after which the specimens are transferred to flow-through baskets for the rest of the processing steps in the microwave.

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5. Dehydration/Resin Infiltration:

1 x 50% ETOH or acetone - 40 sec. in the microwave with a temperature restriction of 37°C
1 x 70% ETOH or acetone - 40 sec. in the microwave with a temperature restriction of 37°C
1 x 90% ETOH or acetone - 40 sec. in the microwave with a temperature restriction of 37°C
2 x 100% ETOH or acetone - 40 sec. in the microwave with a temperature restriction of 37°C

1:1 Resin: 100% solvent - 15 min. in the microwave with a temperature restriction of 45°C
2 x 100% Resin - 15 min. ea. in the microwave with a temperature restriction of 45°C

6. Polymerization:

This can be done conventionally (see UV, Cold Cure and Heat from Protocol 1) or in the microwave. In the microwave the specimens are polymerized under water in 00 BEEM® capsules. The microwave steps are:
10 min. with a temperature restriction at 60°C
10 min. with a temperature restriction at 70°C
25 min. with a temperature restriction at 80°C

NOTES:

1. A good source for information on the use of LR White is: Newman, G.R. 1989. LR White Embedding Medium for Colloidal Gold Methods. In: Colloidal Gold - Principles, Methods and Applications, Vol. 2. M.A. Hayat, ed. Academic Press, Inc., New York, pp. 48-71. (#24966)

2. The technical service department at Ted Pella, Inc. can offer valuable insights into routine and microwave processing techniques using LR White. Feel free to give us a call.

3. Based on our experience and that of others we feel that microwave processing techniques yield consistently better results than routine processing. The sample is in the solvent (<2 min.) and resin (<50 min.) for very short times which appear to greatly increase specimen contrast for EM applications (routine or immunocytochemistry). We prefer a microwave polymerization over oven polymerization because there is no noticeable shrinkage of the resin and the cutting and beam properties of LR White appear to be better as well. These observations are based on our extensive workshop experience and user feedback.

4. The use of Parafilm® M during polymerization in BEEM® capsules in the oven or microwave is necessary for uniform results. The temperature in the convection oven must be ≥65°C for best results.

5. We have found LR White to be a good choice for both routine and immunocytochemistry protocols. Dehydration to the 100% solvent step is not necessary. Stopping at the 70% step as shown in Protocol 1, however, is not our favorite choice. If the 70% change is not accurate the resin will turn cloudy on the 1:1 infiltration step. To avoid problems we prefer at least one 80% or 90% change in solvent before going to 1:1 or 2:1 resin: solvent step.

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6. The results of using vacuum in a laboratory microwave oven have been dramatic. It is a new application which appears to significantly improve results during fixation and shorten microwave infiltration times by 85% over our existing microwave protocols.

7. The expiration, or "use by" dates on LR White Resin are applied in response to a European Common Market (EEC) ruling; they have a limited relationship to the actual shelf life of LR White, especially when the catalyst is added by the end user.

Caution:

All methacrylates (acrylics) should be considered hazardous. Direct contact and inhalation should be strongly avoided. While moderately toxic and allergenic, high concentrations may be very harmful to tissue. In addition, the methacrylates are combustible and vapors may be explosive. All acrylics should be stored in airtight vessels and used in chemical fume hoods with the appropriate protective clothing (gloves and goggles). Recent studies show vinyl, latex and nitrile gloves alone are quickly compromised by contact with methacrylates. Recommended gloves are polyvinyl alcohol ("PVA") or Barrier-brand Product No. 81960-81973 and 81990-81995 Gloves with nitrile.

In the event of direct contact with the skin, the affected area should be immediately wiped clean with dry paper towels followed by a thorough washing with soap and water (see MSDS). Never use an organic solvent to clean embedding media components from the skin.

THE ADDITION OF BENZYL PEROXIDE CATALYST TO LR WHITE RESIN SUPPLIED IN UNCATALYSED FORM

The catalyst supplied by Ted Pella, Inc. for addition or our LR White Resin is a form of benzoyl peroxide in a solid solution to render it safe in transit. Recent modifications to the formulation of this agent by our suppliers result in more catalyst being required if the resin is to polymerize as specified. Accordingly, one 500g bottle of LR White Resin now requires 9.9g of catalyst to be added. If bought in pre-measured aliquots from us, one aliquot should be added to each bottle.

As before, the catalyst should be added to resin at room temperature and the resin must be shaken thoroughly, immediately after addition of catalyst. The catalyst will take a full 24 hours at room temperature to dissolve completely. During this time it is most helpful if the bottle can be shaken from time to time. Do not attempt to heat the resin in order to speed the dissolution of the catalyst. Once mixed and fully dissolved the resin must be stored at 4°C to maintain its shelf life. Once catalyzed, LR White's shelf life is twelve months if stored carefully.

A test aliquot should be polymerized at 60°C for 24 hours following addition of catalyst as a quality control measure. In the unlikely occurrence of this not polymerizing satisfactorily, please contact Ted Pella, Inc. for further advice.

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We do not advise catalyzing less than 500g (one full bottle) of LR White Resin as it can be difficult to accurately measure smaller quantities of the reagents.

LR White Resin with insufficient catalyst will normally polymerize eventually by thermal curing, though curing times may be protracted. If polymerized using LR White Accelerator however, it may only cure to a gel.

If the addition of catalyst is performed correctly, you should expect the resin to behave in the same exemplary way customers have come to expect from the LR White family of resins.

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