LR White Embedding Medium

Catalog LR White Medium grade #14380 and #14382

LR White Resin (1981 is an aromatic acrylic resin mixture which was introduced following the work by Causton, Gillett and Germain, 1980)– It is a very low viscosity (8 cps), low toxicity, beam stable resin. It is a hydrophilic embedding medium and the sections of polymerized LR White resin are hydrophilic. This character allows immuno cytochemistry reagents to easily penetrate into the section without the need of etching, (etching sections can effect delicate tissue antigens).

LR White can be polymerized by four different methods:

1. Heat: 60 – 65°C
2. UV irradiation (365 nm wavelength)
3. Chemical with accelerator
4. Microwave

EMS supplies LR White in either the **premixed form - ready to use (Catalyzed LR White)**, which makes it convenient for the end user or the non-catalyzed version with 9.9g of Catalyst which accompanies the LR White resin with instructions showing you how to catalyze the resin prior to use. (Usually it takes 12 hours at room temperature and 12 hours in the refrigerator for catalyzed LR White resin to take effect prior to use)

LR White (catalyzed) should be kept at 4°C or lower. In our experience, the shelf-life of LR White is a minimum of one year @ 4°C, and up to 15 months if stored as low as -80°C. If the resin is frozen, allow it to thaw and reach room temperature prior to use. If the viscosity of the LR White resin changes (becomes thicker), stop and do not use it.

Using LR White for Electron Microscopy Protocols

Fixation

Conventional EM/LM: 2 to 4 hours - recommended Paraformaldehyde (3 – 4%) solution in 0.1M sodium phosphate buffer, pH 7.2. Avoid using Glutaraldehyde alone or Karnovsky's glutaraldehyde/formaldehyde mixture, for this may lead to a patchy stain or some stains not working well.

For Microwave: Place specimen cubes (~1 to 1.5 mm2) in 1.5ml centrifuge tube with ~600 µl 4% Paraformadehyde in Sodium Phosphate buffer pH 7.2. Microwave irradiate the vial for 40 seconds in a cold spot. Let the vial + tissue + buffer sit for an additional 5 minutes allowing for the cooling of the buffer ≤20°C, and then irradiate again the vial + tissue + buffer for another 40 seconds.

For Immunocytochemistry: 2-3 hours – Recommended 4% paraformaldehyde/0.05% Glutaraldeyde EM Grade/0.2% picric acid in 0.1M sodium Phosphate buffer, pH 7.3 [*Smogyi, P and*

Takagi. H (1982). Note: The use of picric acid-paraformaldehdye-glutaraldehyde fixative for correlated light and electron microscopic immunocytochemistry. Neurosciences 7, 1779 – 1783.]

Buffer rinses are needed at least 2 times and 30 minutes each, for all of applications
Except for the microwave, 2 rinses 3 minutes each is done outside the microwave.

Post Fixation

Conventional: 1 – 2% osmium tetroxide in water or buffer for one hour

For dual LM/LM: post fixation with osmium tetroxide should be avoided.

For Immunocytochemistry: post-osmication or "block staining" should not be carried out. However, post fixing the tissue with 1% tannic acid is recommended.

Then the "Addition of phosphotungstic acid (PTA) to ethanol **for dehydration step**, will improve both the ultrastructure and antigenicity of pituitary tissue embedded in LR White**."** [*Yoko Sakai, Masahiro Hosaka, et al. Histology and Cytology, Vol.68 (2005), No. 5 p.337-34*]

Or, post fix in the mix equal volumes of 3% potassium ferricyanide and 2% osmium tetroxide, with a final concentration of 1.5:1 - buffered or un-buffered, to improved the contrast without using osminum tetroxide [*Berryman & Roddewald, 1990. Histochem/cytochem. 38:159 – 70; Berryman et al., 1992. J. Histochem/Cytochem. 40:845-57*]

For Microwave: same procedure as the fixation for microwave above.

Dehydration / Resin Infiltration

For best results – the use of a rotator for this step is recommended

Ethanol, 200 proof (EtOH) is the choice of dehydration agent for LW White.
(*Acetone acts as a radical scavenger in the resin system and traces of acetone left in the tissue at curing can interfere with polymerization*)

Conventional:

50% EtOH/50% water	-	15 min
70% EtOH/30% water	-	15 min
90% EtOH/10% water	-	10 min
100% EtOH	-	10 min
1:1 EtOH:Resin	-	Overnight
100% Resin	-	2 hours
100% Resin	-	1 hour

For Immunocytochemistry:

Even though the tissue may be taken from 70% ethanol into LW White resin no special procedure is necessary. However, due to the fact that the nature of the tissue is not always consistent, we feel it is safer with at least one more change with 80% alcohol before going to resin.

50% EtOH/50% water	-	15 min
70% EtOH/30% water	-	15 min *
80% EtOH/20% Water		10 min
2:1 LR White resin to 70% EtOH		1 hour**
100% LR White resin		1 hour
100% LR White resin		overnight
100% LR White resin		30 min
100% LR White resin		30 min

* *For enhancing contrast: add 2% PTA to this step, if post fix tissue have been treated with 1% TA.*
***This step is needed to avoid the tissue shrinkage due to the omitted use of osmium tetroxide during fixation.*

For Microwave:

50% EtOH/50% water	-	40 sec with temperature restriction of 37°C
70% EtOH/30% water	-	40 sec with temperature restriction of 37°C
90% EtOH/10% Water		40 sec with temperature restriction of 37°C
100% EtOH		40 sec with temperature restriction of 37°C
100% EtOH		40 sec with temperature restriction of 37°C
1:1 EtOH:LR White resin		15 min with temperature restriction of 45°C
100% LR White resin		15 min with temperature restriction of 45°C
100% LR White resin		15 min with temperature restriction of 45°C

Polymerization

Anaerobic polymerization is advised for curing LR White resin.

Thermal (Heat) Cure: Oven temperature is set at 65°C

If flat embedding is needed for easy orientation of the tissue, we recommend that you should use our PTFE flat embedding mold instead of silicone. (EMS #70085). Resin must be 'over-filled' in the cavities then, cut a piece of paraffin, Aclar film, or polyethylene-based film as big as the mold, and place this film right on top of the resin-filled mold to exclude oxygen.

If BEEM capsules are being used, the lid is removed from the capsule. Filled the capsule and top-off with LR White resin, then use the same method as described above for flat embedding to cover the BEEM capsule.

If gelatin capsules are being used, after fill the capsule with LR White resin, close the capsule and set the temperature at about 55°C (gelatin has a low melting point).

Typical curing time for all the above is overnight to 48 hours, depending on conditions.

Cold cure (use accelerator):

Add 1 drop of the accelerator (10 ml bottle, EMS #14385) to 10 ml of LR White resin and mix well. This mixture will polymerize in less than an hour. However this mixture has very poor infiltration. The resin can be cured in gelatin capsules (EMS #70100), BEEM capsule (EMS #70020), Molding cup tray (EMS #70176-10 series) Peel Away Disposable Mold (EMS #70180 series).

Microwave Cure:

Use BEEM capsule for microwave curing. In a microwave , LR White resin will polymerize under water.

Load the BEEM capsules into a Capsule Holder (EMS #70023). Place the specimen down into the tip of the capsule. Fill the capsules with LR White resin. Put the cap on the capsules (and follow the instruction of this type of capsule holder to secure the capsule lid, so they won't pop out during the polymerization process). Place the whole set into the tray (EMS #97091), fill the tray with water to cover the capsules (approx 350 ml). Place it inside the microwave and start irradiating the resin as follows:

- 10 minutes at a temperature of 60°C* @ full power
- 10 minutes at a temperature of 70°C* @ full power
- 24 minutes at a temperature of 80°C* @ full power
- Let the resin block cool down completely before sectioning.

*Make sure the water is at the requested temperature prior to the run

Trimming and Cutting

When gelatin capsules are used, an initial trimming of the block with a jewelers saw is faster and a final trim with the razor blade is then much easier.

Otherwise, trimming and sectioning of a LR White block may be performed the same way as an epoxy resin block. Glass knives and or diamond knives are routinely used. Cutting speed of about 1mm per second with the thickness of 50 – 70 nm is suitable.

For LM, sections of 2 – 3 µm is nicely obtainable (up to 15 or 20 µm if required)

Section Staining

EM – All common stains give good results on tissue embedded in LR White resin.

As an alternative to uranyl acetate, 1% phosphotungstic acid has been proven to be a good general purpose stain, both as a block stain as mentioned earlier , and as a section stain. The use of ethanol and methanol to make up stains should be avoided, because these solvents will soften the cured resin and may remove sections from supporting grids.

IMMUNOCYTOCHEMISTRY – The choice of immunolocalization technique is entirely up to the user, and PAP, hapten-anti-hapten, avidin-biotin, or gold-colloid methods may be adaptable. (See some helpful references listed below)

Safety

LR White is a low toxicity resin. However all acrylic resins should be considered hazardous. Work under the fume hood, along with safety gloves and goggles are always good laboratory practices. Double-gloves are sometime needed if you are in prolonged direct contact with the methacrylate resins. All chemicals including acrylic resin, should always be tightly closed after each use and stored in a safe place.

If in case you have direct contact with skin or eyes, wash the affected areas with plenty of water and soap.

References

Fixation

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