PROVIDING SOLUTIONS:

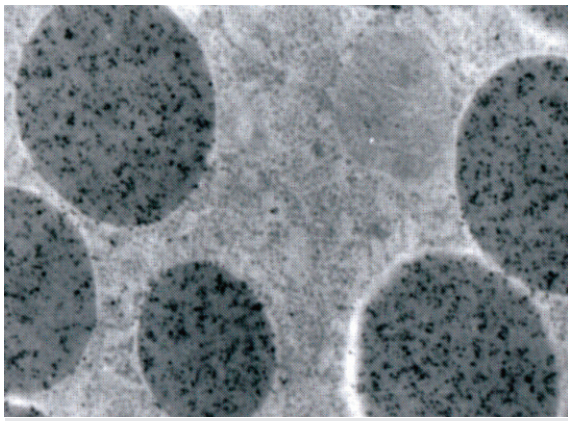
TEM Embedding Resins

KIRSCH NOTES
PROTOCOLS & TIPS FOR MICROSCOPY

Embedding adds desired strength and support to tissue for thin sectioning. It also adds size to specimens for easy handling. Knowing the different properties of several embedding mediums can aid in determining what type of resin would be most suitable for the type of specimen being examined.

Desirable properties for all embedding mediums:

1. Easily available
2. Uniformity from one batch to another
3. Solubility in dehydrating agents
4. Low viscosity as a monomer
5. Uniform polymerization
6. Little volume change on polymerization
7. Ease of sectioning
8. Stability under electron beam
9. Doesn't extract cell constituents
10. Good chromatic (OLM) stainability
11. Low e-scattering = Contrast
12. Good preservation of fine structure
13. Low background granularity



Alpha-amylase on Lowicryl HM20 section of rat pancreas.

The Three Main Types of Embedding Resins

1. Epoxy Resins

Epoxy resins are most widely used since they have most of the desired properties.

Epoxy : Anhydride ratio with higher ratio creating more linear short chain polymers for better cutting.

Weight per Epoxide (WPE): # grams of resin per 1 gram equivalent of epoxide.

Physical Appearance: Transparent, yellowish

Desirable Characteristics

1. Range from viscous liquids to fusible solids depending on the molecular weight
2. Low shrinkage (<2%)
3. Even polymerization
4. Relatively stable with respect to light, heat, and oxygen
5. Relatively stable under electron bombardment
6. Lose only ~25% of the mass of a section under normal operating conditions
7. Can be minimized by exposing the section first to a low intensity beam and then to a gradually more intense beam
8. Maintains three-dimensional structure of the tissue specimens.

Undesirable Characteristics

1. High viscosity
2. Causes severe irritation on prolonged or repeated contact
3. Some (small) loss of contrast between tissue and background

Some Specific Resins

	Cat. No.
Spurr's	**14300** (kit)
Low viscosity (60 cps), toxic; accelerator BDMA. ETOH soluble, poor staining, more shrinkage	
EMbed 812	**14120** (kit)
Uniformity viscosity (150–200 cps) Hardness can be modified by altering DDSA : MNA ratio with a higher portion of MNA making a harder block with a 1:1 ratio providing a very satisfactory hardness. Hardeners: MNA/NMA, Flexibilizer: DDSA, Optional Plasticizer: DBP, Accelerators: DMP-30, DMP-10, BDMA	
Araldite	**13920**
High viscosity (502: 3,000cps, 6005: 1,400 cps) ETOH soluble	
EMbed/Araldite	**13940** (kit)
An excellent sectioning and staining with lower viscosity than 100% Araldite	
Durcupan	**14020** (kit 260 g)
Water soluble, low viscosity polyepoxide with very little shrinkage.	
Durcupan ACM	**14040** (kit 1200 ml)
2 part polyepoxide with low viscosity and very little shrinkage	
Epo-Fix	**1232** (kit)
Originally used for metallographic/materials cold mounting but very good sectioning properties, low viscosity, and very little shrinkage has made it a valuable medium for fibers, papers, powders, and other materials designated for thick and/or thin sectioning. Low exothermic RT polymerization (8 hr.) or 2 hr. at 60° C.	

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2. Methacrylate (Acrylic Resins)

Methacrylate (acrylic resins) are used extensively for immunocytochemistry. They polymerize unevenly and are unstable under electron bombardment.

Physical appearance: Colorless, transparent

Desirable Characteristics

1. Large size sections easily cut
2. Stain readily with excellent contrast obtainable
3. UV polymerization — no OsO_4

Undesirable Characteristics

1. Uneven polymerization
2. Too much shrinkage (15-20%)
3. Reacts with O_2
4. Resins are powerful lipid solvents. Cannot use acetone
5. Hardened resin lacks stability under electron bombardment
6. 50% of mass (may be) lost on electron irradiation followed by flow of remaining resin resulting in distortion of macromolecular structure of tissue.
7. More difficult to section

Some Specific Resins	Cat. No.
L.R. Gold (OLM histochemistry)	**14370**
LR White (EM)	
Very low viscosity, available in different hardness grades:	
Soft	**14384**
Medium	**14381**
Hard	**14383**
Lowicryl (EM)	
High cross-linking, low viscosity. Excellent for immunocytochemical work K4M (-35˚ C)	**14330**
1-step kit	**14335**
HM20 (-70˚ C)	**14340**
HM20 1-step kit	**14345**
KM11 (-60˚ C)	**14350**
HM23 (-80˚ C)	**14360**

3. Polyester Resins

Polyester resins are similar to epoxy resins. They have excellent properties but are usually less easily available, however some components are not stable during storage.

NOTE: Initiator medium contains Benzoyl peroxide and Cobalt Naphthenate (CN) accelerators which when mixed together may cause explosion.

Polymerize by: Light, heat, O_2

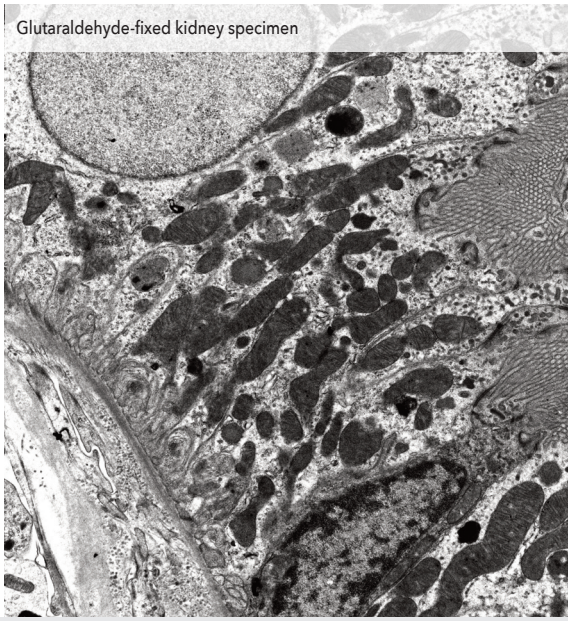
Physical appearance: Colorless, transparent

Some Specific Resins	Cat. No.
Vestopal W — Stains easily	
Rigolac	
Styrene monomer	**14650**
Selectron — First embedment	
Beetle — Rapid embedment	

Technical Tip: Embedding Resin Stain Permeability

Polyester embedding resins are more permeable to acid stains. Epoxy embedding resins are more permeable to basic stains.

Reference: Bryant V. and Watson, J.H.L. (1967). *A comparison of light microscopy staining methods applied to a polyester and three epoxy resins*. Henry Ford Hosp. Med. Bull., 15:65


Glutaraldehyde-fixed kidney specimen

References

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