EMS Microscopy Academy

Endless Possibilities ...

Kirsch Notes

Providing Solutions: TEM Embedding Resins

Embedding Resins

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. Polymerize uniformly
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

EMS Catalog Supplies Mentioned

KITS:

Spurr's	EMS # 14300 (kit)
EMbed 812	EMS# 14120 (kit)
Araldite	EMS# 13920
EMbed/Araldite	EMS# 13940 (kit)
Durcupan	EMS# 14020 (kit 260 g)
Durcupan ACM	EMS# 14040 (kit 1200 ml)
Epo-Fix	EMS# 1232 (kit)

L.R. Gold (OLM histochemistry) EMS# 14370

LR White (EM)

Soft	EMS# 14384
Medium	EMS# 14381
Hard	EMS# 14383

Lowicryl (EM)

	EMS# 14330	1 step kit #14335
HM20 (-70°C)	EMS# 14340	1 step kit #14345
KM11 (-60°C)	EMS# 14350	
HM23 (-80°C)	EMS#14360	

Styrene monomer EMS# 14650

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
General Characteristics:

Desirable Characteristics

- Range from viscous liquids to fusible solids depending on the molecular weight
- Low shrinkage (<2%)
- Even polymerization
- Relatively stable with respect to light, heat, and oxygen
- Relatively stable under electron bombardment
- Lose only ~25% of the mass of a section under normal operating conditions

- Can be minimized by exposing the section first to a low intensity beam and then to a gradually more intense beam
- Maintains three-dimensional structure of the tissue specimens.

Undesirable Characteristics

- High viscosity
- Causes severe irritation on prolonged or repeated contact
- Some (Small) loss of contrast between tissue and background

Some Specific Resins:

Spurr's EMS # 14300 (kit)
Low viscosity (60 cps), toxic; accelerator BDMA.
ETOH soluble, poor staining, more shrinkage

EMbed 812 EMS# 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 EMS# 13920
High viscosity (502- 3,000cps, 6005-1,400 cps) ETOH soluble

EMbed/Araldite EMS# 13940 (kit)
An excellent sectioning and staining with lower viscosity than 100% Araldite

Durcupan EMS# 14020 (kit 260 g)
Water soluble, low viscosity polyepoxide with very little shrinkage.

Durcupan ACM EMS# 14040 (kit 1200 ml)
2 part polyepoxide with low viscosity and very little shrinkage

Epo-Fix EMS# 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.

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
General Characteristics:

Desirable Characteristics

- Large size sections easily cut
- Stain readily with excellent contrast obtainable
- UV Polymerization—No OsO4

Undesirable Characteristics

- Uneven polymerization
- Too much shrinkage (15-20%)
- Reacts with O2
- Resins are powerful lipid solvents. Cannot use acetone
- Hardened resin lacks stability under electron bombardment
- 50% of mass (may be) lost on electron irradiation followed by flow of remaining resin resulting in distortion of macromolecular structure of tissue.
- More difficult to section

Some Specific Resins:

L.R. Gold (OLM histochemistry) EMS# 14370

LR White (EM) – Very low viscosity, available in different hardness grades.

Soft	EMS# 14384
Medium	EMS# 14381
Hard	EMS# 14383

Lowicryl (EM) High cross-linking, low viscosity

Excellent for immunocytochemical work K4M (-35°C)
EMS# 14330 1 step kit #14335

HM20 (-70°C)	EMS# 14340 1 step kit #14345
KM11 (-60°C)	EMS# 14350
HM23 (-80°C)	EMS#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

Vestopal W—Easily stains
Rigolac
Styrene monomer – EMS# 14650
Selectron—First embedment
Beetle—Rapid embedment

References

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Hayat, M.A.1981. *Principles and Techniques of Electron Microscopy*, vol. 1, 2nd ed., pp. 154 -170. Baltimore: University Park Press.

Bozzola, John J. 1999. *Electron Microscopy: Principles and Techniques for Biologists*, 2nd ed, pp. 36-43