EMS Microscopy Academy

Endless Possibilities ...

Kirsch notes — Providing Solutions: Resins

Challenges with Resins

Every step in preparation of samples for TEM is the most critical step. Ensure that you give the same level of attention to each of them:

Fixation Dehydration Infiltration Embedment

A sample may be well fixed and present beautiful morphological features, but if blocks are unsectionable – either too hard or not completely polymerized – or have holes in the tissue, then the sample is ruined. Here we address the most common issues surrounding epoxy resins:

1. Blocks that are too hard
2. Holes in the section
3. Incomplete polymerization/gummy resin
4. Improper polymerization

Epoxy Resin Issues

1 Blocks that are too Hard

Increase the percentage of *Flexibilizer* in the resin recipe.

Embed 812 Recipe

812	50%
DDSA	30%
NMA	20%
DMP-30	1.5 – 2.0%

Spurr's Recipe

ERL 4221	25%
DER 736	25%
NSA	50%
DMAE	0.8%

DER 332-732 Recipe

DER 332	45%
DER 732	20%
DDSA	35%
DMP-30	2%

Araldite Recipe

DDSA	60%
Araldite 6005	40%
BDMA	2.5 - 3.0%

EMS Catalog Supplies

KITS:

EMbed 812 – Catalog #14120
Araldite EMbed 812 – Catalog #13940
Spurr's – Catalog #14300
DER 332-732 – Catalog #14000
Araldite 6005 – Catalog #13920
Lowicryl K4M – Catalog #14330
Glyco-Methacrylate – Catalog #14200
LR White (Medium Grade) – Catalog #14380

NOTE: Araldite 6005 is very viscous before polymerization and a very hard block after. This resin should only be used with hard samples when infiltration is not an issue.

NOTE: There are 3 different accelerators used in epoxy embedding resins, BDMA, DMAE, and DMP-30. Each one can be substituted for the other with BDMA having the lowest viscosity. The amount added to the final total mass are as follows:

BDMA	2.5 – 3.0%
DMP-30	1.5 – 2.0%
DMAE	0.6 – 0.8%

2 Holes in Sections

Holes in sections are caused by:

- Poor infiltration caused by residual water in the tissue after incomplete dehydration.
- Incomplete polymerization caused by moisture in tissue, dehydrant, and/or resin.
- Large defects can be caused by residual trimming artifacts which may cause some pulling out of some cellular components.

3 Incomplete Polymerization/Gummy Resin

Incomplete polymerization or gummy resin is often caused by the presence of residual dehydrant in the tissue, especially when ETOH is used. To avoid this, utilize propylene oxide after ETOH dehydrant for resin infiltration. Glass distilled acetone is often used but can act as a scavenger. Finally, ensure complete infiltration with 100% resin.

For successful embedment follow the following hints:

- Be sure to use fresh or securely sealed bottles of dehydrants.
- Use the purest dehydrants for the final dehydration and infiltration steps: 200 proof ETOH (not denatured) and glass distilled acetone or propylene oxide.
- Be aware of relative humidity in your lab. All dehydrants and resins are hygroscopic and will quickly absorb moisture.
- Be very accurate when measuring the accelerator for your resin, as excess accelerator will make the block harder and brittle.

Methacrylates/Acrylic Resins

4 Improper Polymerization

For Methacrylates or Acrylic Resins, such as LR White, Gold, or Lowicryl

- Acrylic resins react with O_2 so must be polymerized using gelatin capsules or Teflon molds covered with parafilm or some material which is non-permeable to gases. NOTE: **DO NOT** use BEEM capsules, as they are gas-permeable!
- Acetone can not be used for dehydration when acrylic resins are to be used.
- Polymerization is an exothermic reaction and left exposed will generate enough heat to compromise immunocytochemical reactions. Polymerize in 50-60°C oven or UV.