

Laboratory for Advanced Electron and Light Optical Methods

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Resin Formulation

(For further information, see Dykstra, M.J. and L.E. Reuss. 2003. Biological Electron Microscopy. Theory, Techniques and Troubleshooting, 2nd Edition. Kluwer Academic/Plenum Publishers, New York)

General Cautionary Notes For Resin Formulation:

Caution: The chemicals to be mixed can cause contact dermatitis. People with allergies should exercise particular caution. Wear neoprene or latex gloves when working with these components, but recognize **gloves do not confer complete protection from these chemicals**. Work under a fume hood as much as possible. Vinyl cyclohexene dioxide (a component of Spurr resin) is a **known carcinogen**. **Do Not Inhale** resin vapors or allow **skin contact**.

Dispose of resins in **waste resin jar** in the fume hood or polymerize the resin to a solid state before disposal. **Never pour resins down the drain**. Always wipe lip and cap of bottle before reclosing it.

It is far more accurate to measure by weight than by volume. Take a predried 100 ml Nalgene bottle and add the components carefully, in the order given. After all ingredients have been added, cap the bottle. Mix by inversion for a few minutes.

- I. **Spurr's Low-Viscosity Embedding Medium** (Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31.
 - A. **Purpose:** This resin formulation, with a viscosity of 60 cp, was developed for infiltration of plant cells, which are typically harder to penetrate than mammalian cells due to their cell walls. Because of its low viscosity, infiltration times are relatively short, no specimen rotation is necessary, and almost all samples are well-penetrated with our protocols. The resin is **completely miscible** with ethanol, and acetone, though blocks polymerized following an ethanol-only dehydration series often have a slightly tacky surface, unlike those that have been passed through acetone as a transitional solvent. The ethanol-only blocks may also be slightly more brittle during trimming and sectioning. Spurr resin is chemically reactive with silicone flat-embedding molds and eventually ruins them, unlike other epoxide resins.
 - B. **Procedure:** Our recipe is modified from those provided by most vendors supplying the resin components. We have selected the 6.3 g DER recipe as a compromise allowing the polymerized blocks to be equally well-sectioned with glass or diamond knives. If only using glass knives, 6.4 g of DER is the better choice, and if only diamond knives are used, the harder 6.2 g DER recipe is recommended.

1. Add the following components, in the order listed, to a 100 ml Nalgene bottle:

ERL 4221*	10.0 g
D.E.R. 736	6.3 g
NSA	26.0 g
DMAE	0.4 g

*ERL 4221 has replaced the carcinogenic component, vinylcyclohexene dioxide, used prior to the year 2005

Polymerize samples after infiltration at approximately 70°C for 8 hours to 3 days.

Note: Excess resin can be stored by wrapping the Nalgene bottle cap with parafilm and putting in the freezer. It will keep several months. **Always bring to room temperature** before opening to prevent condensation of water on the resin, as it will prevent proper polymerization.

II. **SPI-PON 812, a Substitute for EPON 812, which was discontinued by SHELL Oil Co. in 1978**

Purpose: This resin mixture is supposed to be a substitute for EPON 812. This resin has a higher viscosity than Spurr resin, so it needs a longer infiltration schedule than Spurr resin. In addition, it polymerizes at a lower temperature than Spurr resin and is not reactive with silicone molds, unlike Spurr resin, which is reactive. The plastic trims and sections easily. SPI-PON stains more readily than Spurr and has a slightly grainy appearance. SPI-PON can be stained with 2% aqueous UrAc for about 5 min and for about 8 min in Reynolds' lead citrate to achieve wrinkle-free sections that are a little sharper than Spurr sections stained with methanolic UrAc and lead citrate.

A. **Procedure:**

1. **Resin Formulation:** Add the components **in the order listed**, with mixing.

<u>By Weight</u>	<u>By Volume</u>
18.5 g SPI-PON 812	16.2 ml
9.3 g DDSA	10 ml
10.0 g NMA	8.9 ml
0.6 g DMP-30	0.6 ml

The mixture stays usable for about 1 day at room temperature and will remain usable if frozen for up to 6 months. **Be sure** to cap the storage container tightly and to seal container with Parafilm. After freezing **be sure** to bring to room temperature before opening container. **Water will ruin the resin.**

2. **Sample Processing:** Everything in the embedding and fixation procedure is the same as with Spurr resin procedures except for the embedding procedure outlined below:

- a. After fixation and dehydration to 100% acetone as described in **SOP 6.0.1**, place the samples in in a 1:1 mixture of SPI-PON 812/Acetone at room temperature for about 1 hr with gentle stirring.
- b. Put in 100% SPI-PON 812 for about 1 hr with stirring.
- c. Put in new 100% SPI-PON 812 for about 1 hr with stirring.
- d. Put in 100% SPI-PON 812 in molds and cure at approximately 60° C for about 24 hrs.
- e. Cool to room temperature and section.