

# SPI Chem™ Low Viscosity “Spurr” Kits



## Use Instructions

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### Caution:

Thoroughly read all instructions and warnings on container labels prior to use of this kit.

The Spurr<sup>1</sup> low viscosity system of embedding offers superior penetration of sample material. Among its other convenience features are:

- Lower viscosity than any of the Epon<sup>®</sup> or Araldite<sup>®</sup> resin kits
- Ease of mixing
- Long pot\* life
- Short cure time
- controllable hardness
- Ease of sectioning (glass or diamond knives)

\*Minimal cross-linking and/or self-polymerization of the resin mix at ambient temperatures.

Ultra-thin sections are stable in the electron beam and usually can be mounted on 200 mesh grids without a supporting film. Sections from biological specimens stain readily with uranyl acetate and lead citrate solutions. Good results have also been obtained with phosphotungstic acid stains.

The *original* SPI-Chem™ Spurr Low Viscosity Formulation was based on four components but note that the currently offered kit, which is based on ERL 4221 instead of ERL 4206 is described later on this page:

1. Vinylcyclohexene Dioxide (ERL 4221), a cycloaliphatic diepoxide with a molecular weight of 252.3 and an epoxy equivalent (WPE) of 128 - 145.
2. Diglycidyl Ether of Polypropylene Glycol (DER 736), the flexibilizer, has a WPE of 175-205. The hardness of the cured resin is controlled by varying the amount of DER 736.
3. Nonenyl Succinic Anhydride (NSA), the hardener, has a molecular weight of 224. Exposure to atmospheric moisture must be kept at absolute minimum.
4. Dimethylaminoethanol (DMAE), the recommended cure accelerator, gives a longer pot life to the resin mixture. Curing time can be altered by varying the amount of DMAE used. For faster curing, use Benzyldimethylamine (BDMA) or DMP-30.

Because of safety if not also other concerns, the manufacturer of ERL 4206 discontinued its production in 2003. We do know that some firms have been continuing to offer bottles labelled as ERL 4206 but to the best we can determine, what is actually being sold is ERL 4221 or some other chemical compound.. ERL-4221 is vinyl cyclohexene dioxide.

EPON® - Product of the Shell Chemical Company

ARALDITE® - Product of the Ciba Products Corporation

SPI-Chem™ - Is a trade name of the SPI Supplies Division of Structure Probe, Inc.

Formulations:

The modified (ERL 4221) embedding formulation is shown in the following table:

Weight of Spurr Components in grams for standard (firm) blocks	
Component	Weight, g
ERL 4221	20
DER 736	16
NSA	50
DMAE	0.6

Mixing of components using ERL 4221 formulation:

Procedure to Prepare the Standard Formula (Firm)

The preparation and mixing of the resin components must be thorough and always performed in a fume hood.

1. Weigh a tared disposable plastic beaker and add 86.6 g to the beam (total weight of components to be used).
2. Slowly pour 50 g of NSA into the beaker, adding the last few drops with a spatula or stirring rod.
3. Add 20 g of ERL 4221 to the beaker from a disposable syringe.
4. Add 16 g of DER 736 to the beaker from a disposable syringe.
5. Finally, add 0.6 g of DMAE to the beaker, remove from balance and immediately begin to stir for approximately 3 minutes.

6. Place beaker in a desiccator. This will allow the mixture to free itself of bubbles and mix more thoroughly.
7. In the event of shortened intervals, place the mixture in a vacuum and cycle it to atmosphere until bubbles are removed.
8. Because of the long pot life (3-4 days), the mixture may be prepared the day before use.

To prepare larger amounts, weigh the components into a flask in the manner described (Steps 1-7), stopper and shake the flask vigorously at the appropriate intervals until thoroughly mixed.

The mixed embedding medium can be frozen for several months, and protected from moisture by storing in capped disposable syringes which are then placed in a sealed container with indicating silica gel.

"Original embedding formulations based on ERL 4206" are shown in the following table:

Weight of Spurr Components			
Component			
"Original Formula"	Soft	Firm	Hard
ERL 4206	10.0	10.0	10.0
DER 736	6.0	4.0	7.0
NSA	26.0	26.0	26.0
DMAE	0.4	0.4	0.4

Mixing of resin components (based on use of ERL 4206):

Procedure to Prepare the Standard Formula Firm

The preparation and mixing of the resin components must be thorough and always performed in a fume hood.

1. Weigh a tared disposable plastic beaker and add 42.4 grams to the beam (total weight of components to be used).
2. Slowly pour 26 grams of NSA into the beaker, adding the last few drops with a spatula or stirring rod.
3. Add 10 grams of ERL 4206 to the beaker from a disposable syringe.
4. Add 6 grams of DER 736 to the beaker from a disposable syringe.
5. Finally, add 4 gram of DMAE to the beaker, remove from balance and immediately begin to stir for approximately 3 minutes.
6. Place beaker in a desiccator. This will allow the mixture to free itself of bubbles and mix more thoroughly.
7. In the event of shortened intervals, place the mixture in a vacuum and cycle it to atmosphere until bubbles are removed.
8. Because of the long pot life (3-4 days), the mixture may be prepared the day before use.

To prepare larger amounts, weigh the components into a flask in the manner described (Steps 1-7), stopper and shake the flask vigorously at the appropriate intervals until thoroughly mixed.

The mixed embedding medium can be frozen for several months, and protected from moisture by storing in capped disposable syringes which are then placed in a sealed container with indicating silica gel.

Preparation of the sample for embedding:

Dehydration:

Dehydrate specimens using a graded series of dehydrating fluids: Ethanol, Methanol, Acetone, Tert-Butyl Alcohol, Dioxane, Propylene Oxide, iso-Propyl Alcohol or Hexylene Glycol can be used. These fluids must be anhydrous and totally free of moisture.

Infiltration:

Infiltrate using the complete resin mixture. Continuously agitate the mixture either on a rotator or laboratory shaker during infiltration at room temperature. The changeover from dehydrating fluid to embedding resin can be carried out in several changes of the complete resin, or by dilutions of the resin in the dehydration fluid with soaks at appropriate intervals to bring it to 100% resin mix condition. For larger specimens, infiltrate as described above and soak overnight at room temperature in 100% embedding medium.

Embedding:

Place the sample either in a BEEM<sup>®</sup> flat embedding mold or an SPI Supplies silicone embedding mold or even gelatin capsules containing the Spurr medium and orient, if desired.

An identification code can also be added to the embedding medium on the mold or capsule.

**Cure:**

Set oven carefully and equilibrate at 70°C. Gently place embedments in the oven to cure overnight (8-16 hours).

**References:**

Spurr, A.R., J. Ultrastructure Res.,26, 311 (1969)

**Disclaimer:**

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