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Embed-It™ Low Viscosity Epoxy Kit

A Modified Spurr's Formula For Biological, Material, and Mineralogical Specimen

Background

The introduction of Spurr[1] resin in the early 1960's provided electron microscopists with a very low viscosity resin that gave exceptional penetration qualities. The viscosity of the Spurr resin formulation at 60cps is much lower than other epoxy resins. It readily penetrates membrane walls, hard tissue, rocks, and other minerals. It has been used successfully in the preparation of specimen with high lipid content, tissues with hard lignified walls, and highly vacuolated parenchymatous tissue[2].

Spurr resin is based on a four component mixture. The original formulation was established on a Union Carbide (now Dow Chemical) product, ERL-4206 or VCD. This product is no longer available and has been substituted with ERL-4221. ERL-4221 has worked well, but is significantly higher in viscosity than ERL-4206. High initial viscosity can be detrimental in protocols for geological samples dried by a vacuum displacement process. Dense biological specimen often require a low viscosity resin for adequate penetration during infiltration. While a four-part system offers some latitude in controlling or altering the properties of the resin, it takes more time and opens up room for greater error and inconsistency. This can result in loss of valuable specimen.

Polysciences' new *Embed-It* Low Viscosity Epoxy Kit consists of two very low viscosity (~65cps) liquids which are mixed in equal parts by weight. The mixed components build slowly in viscosity over a 48-hour period to about 500cps. The material cures to a hard solid overnight at 60°C or in about 10 hours at 70°C. Thin sections may be cured faster at higher temperatures, however there is the possibility of the material darkening due to higher exothermic temperatures.

The new *Embed-It* Low Viscosity Epoxy Resin provides the same results as the original Spurr Low Viscosity Resin with a number of advantages.

- Clear blocks enable easy tissue location.
- **Easy to Use** - mix only 2 components in equal proportion
- **Save Time** - use same solution for both infiltration and embedding
- **Convenient** - mix only the amount you need
- **Less Hazardous** - components shipped together as non-hazardous materials
- **Stable** - product has excellent stability

Fixation

Specimens can be fixed in formaldehyde (paraformaldehyde) and glutaraldehyde solutions as required by the laboratory. Post fixation in osmium tetroxide can be completed prior to infiltration. Non-biological specimens may not require a fixation step.

Dehydration

Dehydration must be completed for all specimens. Failure to do so will result in unpolymerized portions of the block. The specimens can be dehydrated with Ethanol or Acetone to 100% to remove moisture. Propylene Oxide is not required for dehydration. The time required for each dehydration step will depend on the size of the specimen and laboratory protocol.

Mineralogical and material science specimens may require only drying prior to infiltration and embedding procedures.

Mixing and Infiltration

The *Embed-It* Low Viscosity Epoxy Resin is used for both infiltration and embedding. To prepare the *Embed-It* Resin, mix together equal parts, by weight, of *Embed-It* Solution A and *Embed-It* Solution B. For example, mix 2gms of *Embed-It* Solution A with 2gms of *Embed-It* Solution B. Because the components in the *Embed-It* Low Viscosity Epoxy Kit are mixed one to one, mix only the amount required as needed.

The *Embed-It* Resin can be divided for infiltration with mixtures of the final dehydrant. Infiltration for dried specimen can be started directly with *Embed-It* Resin. These steps are generally 10 minutes to several hours depending on specimen type and size.

1. 1 part *Embed-It* Resin to 3 parts dehydrant
2. 1 part *Embed-It* Resin to 2 parts dehydrant
3. 1 part *Embed-It* Resin to 1 part dehydrant
4. *Embed-It* Resin alone will be a minimum of two changes to replace any excess dehydrant in the specimen. The final step in *Embed-It* Resin should be several hours to overnight.

Embedding
The specimen can be removed from the last step of infiltration and oriented in the mold of choice. Once the specimen is in the correct orientation, fill the mold with *Embed-It* Resin. If embedding in BEEM® capsules or small molds, polymerize overnight in a 60°C oven. Additional time will be required for larger specimen. If the block does not appear to be completely polymerized, it can remain in the oven for the time required to harden the block. The temperature can be raised to 70°C, although increased temperature may darken the blocks.

Sectioning and Staining
Sectioning is completed as usual with glass, diamond, or tungsten carbide knives. The sections can be placed on a slide for staining and block orientation or on grids with the appropriate stains for electron microscopy. Lead citrate and uranyl acetate can be used with excellent results.

Caution
Do not get on skin, clothing, or in eyes. Avoid inhalation of vapors and mists. In the case of contact with eyes or skin, flush immediately with copious amounts of water for at least 15 minutes; for eyes get immediate medical attention. Remove contaminated clothing and shoes at once. This product should be used under a hood at all times during the infiltration and embedding process. The oven required for polymerization should be under a hood or have adequate ventilation. Wear gloves and safety glasses when handling these components. Wash hands immediately after handling.

Ordering Information

Cat. #	Description	Size
24300-1	*Embed-It*™ Low Viscosity Epoxy Kit	1 kit
00236-1	Propylene Oxide, EM Grade	1pint
00236-6	Propylene Oxide, EM Grade	6 x 1pint
01921-4	Acetone, EM Grade, 99.5%	4 x 1L
01921-6	Acetone, EM Grade, 99.5%	6 pints
09860-1	Reagent Grade Alcohol (100%)	1 gallon
00224-100	BEEM® Capsules Size 00	1 pkg of 100
00294-100	BEEM® Capsules Size 00 Conical	1 pkg of 100
00336-100	BEEM® Capsules Size 3	1 pkg of 100
00295-100	BEEM® Capsules Size 00 Bottle Neck	1 pkg of 100
00225-1000	Gelatin, Embedding capsules Size 00 (23.3mm L x 8.18mm W x 0.95mL volume)	1 pkg of 1000
07347-1000	Gelatin, Embedding capsules Size 1 (19.0mm L x 6.63mm W x .50mL volume)	1 pkg of 1000
07348-1000	Gelatin, Embedding capsules Size 3 (13.9mm L x 5.05mm W 0.21mL volume)	1 pkg of 1000
23257-1	BEEM®, Flat, Transparent	1 mold
23256-1	Chien 8 Faced Mold	1 mold
19440-1	Chien Universal Mold	1 mold

Also available from Polysciences, Inc. are a variety of EM stains, reagents, and a wide selection of grids.

To Order

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In Germany Call: (49) 6221-765767
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Order online anytime at **www.polysciences.com**

References
1. Spurr, A.R., J. Ultrastructure Res., 26:31 (1969)
2. Spurr, A.R., and Harris, W.M., Am. J. Botany, 55 (1968).