

Spurr Low-Viscosity Embedding Media

For Biological, Material and Mineralogical Specimen

Background and Advantages

The introduction of Spurr¹ resin provided the microscopist with a resin of exceptional penetration qualities. Unlike the other epoxies Spurr resin with a viscosity of 60 cps will readily penetrate membrane walls, hard tissue, rocks and other minerals. It has also been used successfully in preparations of specimens with a high lipid content, tissues with hard, lignified walls, and highly vacuolated parenchymatous tissue.²

The Spurr resin is based on the four following components. ERL 4221, which because of its compact structure yields highly cross-linked polymers with good high temperature resistance.

The flexibilizer is the epoxy resin, D.E.R. 736, (diglycidyl ether of polypropylene glycol). This has a WPE value of 175-205. The amount of D.E.R. 736 may be regulated to provide embedments with a variety of hardness characteristics. (*see Table 1*)

NSA, Nonenyl succinic anhydride is the hardener specially purified for EM usage. To avoid hydrolysis of epoxide or anhydride bond, we recommend minimum exposure of the above to atmospheric moisture. The accelerator is dimethylaminoethanol (DMAE). This agent gives longer pot life to the resin with a lighter color. Curing times can be shortened by increasing the accelerator ratios. (*see Table 1*)

| Ingredient | Standard Medium | Suggested Modifications of the Medium | |
|---|-----------------|---------------------------------------|-----------|
| | A Firm | B Hard | C Soft |
| ERL 4221 | 4.10gm | 4.10gm | 4.10gm |
| Diglycidyl ether of polypropylene glycol (D.E.R. 736) (d) | 1.43gm | 0.95gm | 1.90gm |
| Nonenyl succinic anhydride (NSA) | 5.90gm | 5.90gm | 5.90gm |
| Dimethylaminoethanol (DMAE) (c) | 0.1gm | 0.1gm | 0.1gm |
| Cure schedule (hr) at 70°C (a) | 8 | 8 | 8 |
| Pot life (days) (b) | 3.4 | 3.4 | 3.4 |

(a) Cure for minimum hours indicated or longer, generally overnight. (b) Time between initial mixing and end point for convenient use, store at room temperature in a closed container. (c) As dimethylaminoethanol increases, color increases. (d) Reducing D.E.R. 736 lightly improves the color.

Preparation:

The medium is easily and rapidly prepared by weighing the components singly into a tared disposable plastic beaker. Exact weights should be used for optimum performance. The DMAE should be added last after *gently* mixing other components. The complete medium should now be mixed thoroughly by stirring. The resin mix should then be placed in a desiccator for storage during specimen dehydration and infiltration time.

The embedding medium should be freshly prepared, but it may be kept for convenience in measured aliquots. These are best stored when sealed, and anhydrous, in a deep freeze without the DMAE.

Dehydration of biological and some mineral specimens is achieved by the usual graded series of dehydrating fluids. The medium is compatible in all preparations with ethanol, methanol, acetone, tert-butyl alcohol, dioxane, hexylene glycol, isopropyl alcohol and propylene oxide. To minimize lipid loss, use water and hexylene glycol at increasing concentrations or ascending concentrations of 10%, 20%, 40%, 60%, 80%, and 2 changes of 100% hexylene glycol. The dehydration process with hexylene glycol has been used successfully by Spurr.

Use the complete medium for infiltration. Continuous mild agitation is desirable during the infiltration using a rotator or shaker. Several variations of the infiltration procedure have been used. In some cases, the changeover from the dehydrating fluid to embedding fluid is made in 3 stages, while in others additional intermediate steps are needed. With larger specimens, it is beneficial to infiltrate in the afternoon and soak overnight in the embedding medium.

A rapid method useful for biopsies³ can be employed which requires only 2 hours to complete embedments. With mineral specimens, alternate vacuum may help to speed impregnation. Embedding may be done with oven-dry gelatin capsules or Polysciences' Flat Embedding Molds. Specimens generally sink to the bottom of the liquid media. Blocks can be cured in 8 hours in a 70°C oven, 16 to 24 hours cures will not damage embedments. If modifications of cure are required, Table 1 provides suggestions.

Another embedding medium, which combines qualities of Poly/Bed[®] 812 and Spurr's mix was recommended.⁴ This media consists of a 1:1 mixture of Spurr's resin and Poly/Bed[®] 812 (Luft formula).⁵ Spurr's resin has also been most recently used as an excellent embedding media for immunohistochemical stains at the light microscopy level.¹⁰

Trimming and Polishing of Embedments:

The castings have good trimming, sectioning and polishing qualities. The block faces are hydrophobic and are not wetted easily by distilled water during sectioning. Sections are tough under the electron beam and can be used without a supporting membrane on a 200 mesh grid. Mineral specimens can be easily polished on a lapping wheel. Castings are relatively inert and are resistant to KMnO_4 or $\text{Ba}(\text{MnO}_4)_2$. No noticeable effect of the electron transmission on the background plastic is observed with electron dense stains.

Grid staining for some tissues with uranyl acetate and lead citrate may require longer periods than usual or mild heat may be employed during staining. En bloc with uranyl acetate is recommended.

The use of hydrogen peroxide to accelerate staining of Spurr sections,⁶ and Seligman Osmiophilic Stain Techniques⁷ work satisfactory. Excellent staining for Light Microscopy has been obtained by the Azur II methylene blue method of Richardson et al.,⁸ and using Polysciences' Multiple Stain.⁹

Handling Precautions:

The toxicological properties of the components of this unit are not fully known. Prolonged and repeated contact of liquid or breathing of vapors or mists of the components used in this kit singly or collectively may cause delayed and serious injury. Do not get on skin, in eyes or on clothing. Avoid inhalation of vapors or mists. In case of contact with eyes, immediately flush with plenty of water for at least 15 minutes; for eyes, get medical attention. In case of skin contact, wash with soap and water. Remove contaminated clothing and shoes at once. Wash thoroughly before re-use. Use only with adequate ventilation. Use of goggles and gloves are recommended.

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Ordering Information:

| Cat. # | Description | Size |
|--|--|----------------|
| 01916 | Spurr Low-Viscosity Embedding Kit | 1 Kit |
| 00224 | Embedding Capsules, BEEM*, size 00 | 1 pkg of 100 |
| Osmium Tetroxide, Crystalline, 99.95% | | |
| 0223A | Osmium tetroxide, crystalline | 5 x 1 g |
| 0223B | Osmium tetroxide, crystalline | 10 x 1 g |
| 0223C | Osmium tetroxide, crystalline | 10 x 1/2 g |
| 0223D | Osmium tetroxide, crystalline | 10 x 1/4 g |
| Osmium Tetroxide, 4% Solution | | |
| 0972A | Osmium tetroxide, 4% solution | 20 x 2 ml amp |
| 0972B | Osmium tetroxide, 4% solution | 5 x 10 ml amp |
| 0972C | Osmium tetroxide, 4% solution | 20 x 10 ml amp |
| Osmium Tetroxide, 2% Solution | | |
| 23310 | Osmium tetroxide, 2% solution | 10 x 2 ml amp |
| 23311 | Osmium tetroxide, 2% solution | 10 x 5 ml amp |
| 21447 | Uranyl Acetate (98% ACS Reagent) | 25 g |
| 00378 | Lead Citrate Trihydrate | 100 g |
| 00216 | Glutaraldehyde, E.M. Grade | 30x10 ml amp |
| 07450 | Disposable Beaker size 50 | 1 box |
| 02615 | Flat Embedding Mold | 1 each |
| 08824 | Multiple Stain Solution | 50 ml |
| 08792 | Poly/Bed® 812 (Luft Formula) Embedding Kit / DMP-30 | 1 Kit |

To Order:

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