

# Araldite EM Media

## Araldite

The Araldites are useful embedding media for Electron Microscopy as they polymerize uniformly with volume shrinkage as low as 1-2%. Araldite resins do not degrade and cause contamination in the microscope due to electron bombardment.

Polysciences provides two Araldite types. Araldite 502 and 6005, which are medium viscosity, are most popular. Araldite 502 is a modified Epoxy Resin containing a nonreactive diluent which increases resiliency of the cured product. This resin corresponds to the British Araldite M, used in original publications.<sup>1,2</sup> Araldite 6005 is an undiluted medium viscosity resin.

The viscosity of Araldites can be reduced by adding dibutyl phthalate.<sup>3</sup> Luft<sup>4</sup> and Mollenhauer<sup>5</sup> later developed modifications to the original technique. All reagents described herein are available from Polysciences. See section on Handling Precautions regarding the use of all materials mentioned.

## Luft Araldite Procedure:

The Luft method overcomes the original difficulties of penetration and sectioning, and permits embedding of tissue from fixation to sectioning in 24-48 hours. His modification uses propylene oxide as an additional clearing stage beyond ethanol and a carefully controlled resin proportion to control the degree of polymeric crosslinkage. The type of tissue to be embedded and the viscosity of the Araldite selected will determine the infiltration time which is performed without the accelerator. Hard tissue or more viscous embedding media require longer treatment to insure a thorough penetration of the tissue. The Araldite embedding mixture is soluble in ethanol and propylene oxide. All glassware should be rinsed immediately after use, since the Araldite is insoluble when polymerized.

## Luft Araldite Resin Mixture:

- 54 ml - Araldite 502
- 46 ml - DDSA, Dodecylsuccinic anhydride (Hardener)
- 1.5-2.0% - DMP-30 Accelerator, [2,4,6-tri(dimethylaminoethyl)phenol] Add the accelerator just before use.

The Araldite 502 resin sold by Polysciences is carefully controlled to have a weight per epoxide equivalent of 233-250. The above mixture has an anhydride-epoxy ratio of 0.7, which is considered by

Luft to give optimum properties. The Araldite-DDSA mixture can be stored up to six months, if refrigerated at 4°C. The cold solution should warm up to room temperature before use to obviate moisture condensation which destroys the anhydride and will lead to poor results. The accelerator, DMP-30, determines the polymerization time, and the amount is critical. It should be added just before use to forestall premature polymerization.

## Luft Technique:

1. Tissues are fixed with Osmium tetroxide (*catalog #0972A*) and glutaraldehyde (*catalog #00216A*), and dehydrated with a graded series of ethyl alcohol (*Catalog #09860*). The alcohol is poured off.
2. Tissue blocks are immersed in two changes of propylene oxide for 10-15 minutes each. (**Avoid inhalation of the vapors.** Evaporation of the liquid may cause condensation on the tissues.) **Propylene oxide is very flammable and should be flushed down the sink with a large excess of water.**
3. Fresh propylene oxide is added to an equal volume of freshly prepared resin mixture plus accelerator, DMP-30 (1.5-2% by volume.) Add accelerator just before use. Mix tissue well by swirling. After one hour, change to a 1 part propylene oxide/3 parts resin mixture. Allow an additional 3-6 hours or longer for infiltration.
4. Fill clean, dry BEEM\* Capsules or flat Polysciences' embedding molds (*Catalog #02615*) with undiluted resin mixture plus accelerator (no propylene oxide) to within 1-2 mm of top. Specimen is transferred to the capsule by pipette with a minimum of retained solvent. Stirring is not necessary.
5. Polymerize according to the following schedule:
  - a. Overnight 35°C
  - b. Next day 45°C
  - c. Next day 60°CSatisfactory results have been obtained overnight at 60°C, but the 3-stage cure is preferred.
6. Cool blocks to room temperature before sectioning. The tissues are easily sectioned and show excellent contrast and preservation. The sections can be self supporting on a 200 mesh or 300 mesh grid.

**Mollenhauer Embedding Method:**

Mollenhauer<sup>4</sup> generated considerable interest by embedding in Araldite-Poly/Bed<sup>®</sup> 812 mixtures. Poly/Bed<sup>®</sup> 812 is Polysciences equivalent of Epon 812. Mollenhauer Mixture containing 100 ml Poly/Bed<sup>®</sup> 812 and 60 ml Araldite 502, produced relatively hard blocks with high image contrast. Private correspondence with Dr. Mollenhauer indicated that this was the most reliable of his mixtures and that tissues embedded therein were easier to section than when embedded in either Poly/Bed<sup>®</sup> 812 or Araldite alone.

**Mollenhauer Mixture 1:**

- 100g Poly/Bed<sup>®</sup> 812
  - 55g Araldite 502 or 6005
  - 180g DDSA, Dodecenylsuccinic anhydride
  - 10g BDMA, Benzyldimethylamine, accelerator\*
- \*(5g of DMP-30 may be substituted for BDMA in the above mixture.) Poly/Bed<sup>®</sup> 812 has an epoxide equivalent of 150±5 and the Araldite 502 has an equivalent of 240±5 to insure optimum results. Deviation may cause difficulties in sectioning.

Other variations of Mollenhauer include the following:

1. 45ml - Araldite, 502 or 6005  
165ml - DDSA  
75ml - Poly/Bed<sup>®</sup> 812  
1.5% - DMP-30 or BDMA 3%  
6-12ml - Dibutyl phthalate
2. 81ml - Araldite 506  
62ml - Poly/Bed<sup>®</sup> 812  
100ml - DDSA  
1.5% - DMP-30 or BDMA 3%  
4-7ml - DBP (Dibutyl phthalate)

Dibutyl phthalate is a plasticizer and also reduces the viscosity of the uncured resin mixture. If the dibutyl phthalate is omitted, a very hard block results.

Dr. E.H. Newcomb<sup>6</sup>:

- 43g - Araldite 6005
- 43g - Poly/Bed<sup>®</sup> 812
- 114g - DDSA

DMP-30, accelerator, is used at the rate of 3 drops per 5ml of embedding resin.

The embedding sequence for the Mollenhauer and Newcomb methods is similar to step 1 and 2 of the Luft procedure. Following these dehydration steps, immerse the tissue in a 2:1 ratio of solvent (propylene oxide) to plastic mixture with accelerator for one hour at room temperature. Repeat immersion in a 1:2 ratio of solvent to plastic mixture with accelerator for one hour at room tem-

perature. Finally soak in an all-plastic mixture with accelerator for 2-4 hours at room temperature and incubate in a 60° oven until hard (usually overnight).

Nonenylsuccinic anhydride, E.M. Grade, when substituted for DDSA, has a lower viscosity and better color. Butanediol diglycidyl ether which is a reactive diluent can be substituted for dibutyl phthalate to cut viscosity of the mixture.

Reference to our standard #233 Poly/Bed<sup>®</sup> 812 and #127 Spurr Data Sheets will provide suggestions for further variations.

**Handling Precautions:**

**Flammable:** Avoid flames, sparks, and heated surfaces. Use only with adequate ventilation in chemical hood. Wear gloves, goggles, and protective clothing.

**Warning:** 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 or vapors or mists. In case of contact with eyes or skin, immediately flush with plenty of water and wash for at least 15 minutes; for eyes, get medical attention. Remove contaminated clothing and shoes at once. Wash thoroughly before re-use.

**References**

1. Glauert, A.M., et al., *Nature*, **178**, 803 (1956).
2. Glauert, A.M., and Glauert, R.H., *J. Biophys. Biochem. Cytol.*, **4**, 191 (1958).
3. Ellis, A., *EMSA Bull.*, **16**, 53 (1986).
4. Luft, J.H., *J. Biophys. Biochem. Cytol.*, **9**, 409 (1961).
5. Mollenhauer, H.H., *Stain Technology*, **39**, 111 (1964).
6. Newcomb, E.H., Botany Dept., Univ. of Wisconsin, Private Communication.

\*\*"BEEM" is a registered Trademark of Better Equipment for Electron Microscopy, Inc.

**Ordering Information:**

<b>Cat. #</b>	<b>Description</b>	<b>Size</b>
02600	Araldite 502, Luft Kit Contains 500g Araldite 502, 450g DDSA, 100g DMP 30.	1kit
02595	Araldite Embedding Kit, Mollenhauer Contains 500g Araldite 502, 500g PolyBed, 450g DDSA, 100g DMP 30.	1kit
00552	Araldite 502	500g
02116	Araldite 6005	500g

**Araldite Accelerators and Hardeners:**

<b>Cat. #</b>	<b>Description</b>	<b>Size</b>
00553	DMP-30	100g
00141	N,N-Benzylidimethylamine	100g 500g
01251	Triethylenetetramine	500g
00563	Dodeceny succinic anhydride (DDSA)	450g 4x450g
00886	Nadic methyl anhydride (NMA)	450g
00224	BEEM* Embedding Capsules, size 00	100 500 1000

**Mini Embedding Kit**

<b>Cat. #</b>	<b>Description</b>	<b>Size</b>
21960	Poly/Bed 812/Araldite 502 Mini Embedding Kit for fewer applications Includes:	
	DDSA	55ml
	Poly/Bed 812	30ml
	Araldite 502	15ml
	DMP-30	2ml
	Total Mix	102ml

3 10ml Storage Syringes, 1 Graduated 3ml Syringe  
2 Pair Protective Gloves, 50 BEEM\* Capsules, 2 Mixers

**To Order:**

In The U.S. Call: 1-800-523-2575 • 215-343-6484

In The U.S. FAX: 1-800-343-3291 • 215-343-0214

In Germany Call: (49) 6221-765767

In Germany FAX: (49) 6221-764620