PELCO TECHNICAL NOTES

	Eponate 12 ™-Araldite 502 Kit With BDMA	
	Prod. No. 18028	
20	Contains:	
	18060 Araldite 502 Resin450g	
	18005 Eponate 12 [™] Resin450g ■ I	
	18022 DDSA 450g ■ I	
	18241 BDMA25g ■ T	
	e	

with DMP-30 Prod. No. 18030				
Contains:				
18060 Araldite 502 Resin450g	Ι			
18005 Eponate 12 TM Resin450g	Ι			
18022 DDSA 450g	Ι			
18042 DMP-3025g	I			

Eponate 12[™]-Araldite 502 Kit

■ I: Irritant; ■ T: Toxic

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Resin: The Epon-Araldite formula devised by Mollenhauer (1964) combines the best qualities of Epon and Araldite. Excellent ultrastructural preservation is retained, yet the blocks section easily and stain readily. The relatively low viscosities of the Eponate 12[™] and Araldite allow good tissue penetration during infiltration steps.

Formulation: A block of MEDIUM hardness can be obtained by using the following formulae (A: $E \cong 0.8$):

Eponate 12	25ml	DDSA	60ml
Araldite 502	15ml	DMP-30	2ml
		or	
		BDMA	3ml

10 **Procedure:** Either ethanol or acetone may be used for dehydration. If ethanol and propylene oxide are used, one should be careful not to remove all the propylene oxide when transferring the specimen, since this volatile liquid can rapidly evaporate and leave the specimen dry.

For infiltration the solvent is replaced with a 1:1 mixture of solvent and embedding medium and gently = infiltrated (mixed) for at least one hour at room temperature. Then pour off or pipette out the 1:1 mixture and replace it with the 100% complete embedding medium for further infiltration of 30-60 minutes, preferably uncovered to allow any remaining solvent to evaporate. A second 30 minute infiltration with the 100% 12 embedding medium is recommended. Specimens are transferred to clean dry capsules, which are then filled н with fresh embedding medium. An overnight polymerization can be obtained at 60° C.²

Suggestion for harder blocks: A harder block can be produced by decreasing the DDSA component in the above formula and substituting Nadic Methyl Anhydride (NMA) for the balance of the formula. However, keep in mind that NMA has a higher anhydride content than DDSA and, therefore, one should add a quantity of NMA equivalent to 50% of the quantity specified for DDSA to maintain an equivalent A:E ratio. In other words, for each 1 ml of DDSA specified, substitute 0.5 ml of NMA.¹

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Suggestion for Mixing: To facilitate mixing, the user may wish to warm the components to 55-60°C prior to combining. If the components are heated in such a manner, only a few minutes of gentle stirring or "rotation" are necessary for adequate mixing.¹

Accelerator: The researcher may wish to substitute BDMA for DMP-30 as the accelerator, since BDMA tends to be less viscous and more uniform with the added feature of a longer shelf life. Many researchers prefer BDMA, since problems of uneven embedment hardness have been linked to inadequate distribution of the accelerator. Dr. Brian Causton (Proc. RMS, 15, 185-189, 1980) reports that the rate of diffusion of DMP-30 is slower than that of BDMA. The recommended concentration of BDMA is 2-3%, as compared to the 1.5-2.0% recommended for DMP-30.¹

Use of Weight-per-epoxide Equivalent (WPE) and Anhydride To Epoxy (A:E) Ratios. Since the WPE value varies from lot to lot for the various epoxies, we recommend utilizing WPE values when formulating the final mixture of epoxy embedding media to help maintain reproducibility of block characteristics, although not all researchers find this consideration significant. The A:E ratio should be approximately 0.75-0.85.¹

Note that if this is not done, the final characteristics of the embedments may vary significantly with the WPE changes produced by the various manufacturing techniques utilized in the production of epoxies.

Technical Data:

	Density	Molecular Weight			
DDSA	1.00g/ml	266			
Araldite 502	1.13g/ml	WPE=222			
	1.17g/ml	WPE=235			
Eponate	1.22g/ml	WPE=145			
	1.15g/ml	WPE=160			
DMP-30.	0.97g/ml	265			
BDMA	0.90-1.10g/ml	135			
Note: the viscosity of Araldite 502 at 25°C is 2100-3600 cP.					

Caution: Most epoxies are suspected carcinogens and therefore should be handled with great care. Epoxies, anhydrides and accelerators should all be considered toxic, in a general sense. Care should be taken to avoid direct contact with liquids or their vapors or dusts produced from the polymerized blocks. All work with these components, or mixtures of components, must be carefully performed within a properly vented fume hood.²

In the event of direct contact with the skin, the affected areas should be immediately wiped dry with clean, dry paper towels, followed by a thorough washing with soap and water. (Never use an organic solvent to clean the skin).

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Hints:

- 1. Warm the resins, hardeners and containers to 60°C for at least 10 minutes. This practice decreases the viscosity of epoxy resins, and has no other effect on them.²
- 2. Measurements by volume are much easier than measurements by weight.³
- 3. Storage of mixtures of epoxy resins and hardeners should be avoided because cross-linking will occur even without the accelerator.
- 4. Moisture must be avoided in these mixtures.
- 5. Never open bottles which have been refrigerated until room temperature is reached.
- 6. Storage of accelerators in a desiccator is helpful. Humidity will gradually deactivate the amine.
- 7. Do not use stirring rods when mixing the resins after the materials have been warmed. A warm conical flask may be used which can be shaken gently for a few minutes until it is evident that mixing is complete.²
- 8. The accelerator BDMA can be dispensed from a graduated pipette or with previously calibrated drops.²
- 9. If a graduate cylinder and conical flask are used, they can be drained immediately after use by inverting them over disposable containers and then reused. No washing is required. Surplus embedding medium collected can then be left to harden and can be disposed of properly.

Note: See PELCO® Technical Note - "Resins and Embedding-General Procedures"

Footnotes:

- 1. Personal Communication, Audrey Glauert.
- 2. Glauert, A. M., ed: <u>Practical Methods in Electron Microscopy. Ro. 143-144</u>. North-Holland American Elsevier, 1975.
- 3. Coulter Hd: <u>Rapid and improved methods for embedding biological tissues in Eponate 12 and Araldite</u> <u>502</u>. J Ultrastruct Res, 20, 346-345, 1967.

References:

Glauert, A. M., ed: <u>Practical Methods in Electron Microscopy. Ro. 143-144</u>. North-Holland American Elsevier, 1975.

Mollenhauer, H. H.: Stain Technology 39:1 i (1964).

Hayat M. A.: <u>Principles and Techniques of Electron Microscopy</u>, 3rd Ed., CRC Press (Boca Raton, FI), p. 94, 1989.

Glauert A. M.: <u>Epoxy resins: an update on their selection and use</u>, Microscopy and Analysis, 15-20, Sept 1991.

Luft J.H.: <u>Improvements in Epoxy Resin Embedding Methods</u>, J Biophys Biochem, Cyto, 9:409-414, 1961. Coulter Hd: <u>Rapid and improved methods for embedding biological tissues in Eponate 12 and Araldite 502</u>. J Ultrastruct Res, 20, 346-345, 1967.

Glauert A. M.: Personal Communication.

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