PELCO® Eponate 12™ Kit
With DMP-30
Product No. 18010

Contains:

18005 Eponate 12 Resin	450g	• I
18022 DDSA	450g	• I
18032 NMA	450g	• C, I
18042 DMP-30	25g	• T

PELCO® Eponate 12™ Kit
with BDMA
Product No. 18012

Contains:

18005 Eponate 12 Resin	450g	• I, Cg
18022 DDSA	450g	• I
18032 NMA	450g	• I, C
18241 BDMA	25g	• I, C, T, F

Resin: Eponate 12T"' is a generic replacement for Epon 812, formerly available from Shell Chemical Co. The resin is a glycerol polyglycidyl ether that can be formulated and used as an embedding medium in exactly the same manner as the old Epon 812 from Shell. The addition of the anhydrides DDSA and NMA makes it possible to mix different formulations that yield cured blocks of a reproducible hardness. *Eponate Kit 18012 wlBDMA accelerator is recommend because ii defuses into tissues more quickly than DMP-30.* 1

Accelerator: The difference between the two kits is the tertiary amine accelerator. Cat. No. 18012 contains BDMA and Cat. No. 18010 contains DMP-30 as the accelerator. BDMA is used at a concentration of 2.5-<3.0%; DMP-30 is used at a concentration range of 1.5-2%. "BDMA is a smaller molecule which penetrates faster, has a lower viscosity and a much longer shelf life than DMP-30."2

NOTE: Using BDMA at the maximum concentration a significant viscosity increase can be expected with overnight filtrations or when the resin mixture is used in an automatic tissue processor and left overnight. A suitable compromise of a lower BDMA concentration may solve this problem.

Curing: Curing is achieved in 16-24 hours at 60°C. Optimum cross-linking will have occurred in this time period. An increase in temperature will facilitate a more rapid cure with increased cross-linking. This will alter the characteristics of the block and its expected trimming and sectioning properties.

One-Mix Formulation: This can be used in place of the A and B mixtures proposed by Luft (3). The WPE of the resin for these formulations is 145.

One Mix Formulations for -50m1 Batch Sizes

Component	SOFT ml	SOFT g	MEDIUM ml	MEDIUM g	HARD ml	HARD g
Resin	22.2	27.0	23.8	29.0	25.7	31.4
DDSA	21.5	21.5	16.0	16.0	9.3	9.3
NMA	6.9	8.5	11.5	14.3	16.5	20.5
Accelerator						
DMP-30 (1.5%) or	0.8	0.8	0.8	0.8	0.8	0.8
BDMA (2.5-3%)	1.6	1.7	1.6	1.7	1.6	1.7

The ratio in moles of NMA:DDSA determines the block hardness. The softest bocks contain no NMA and the hardest blocks contain no DDSA. The A:E ratio for these formulations is 0.7:1 and represents the molar ratio of anhydrides to resin in moles. The WIPE equals the molecular weight for the resin.

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. 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.)

Technical Data:	**Density**	**Molecular Weight**
DDSA	1.00G/ML	266
NMA	1.24g/ml	178
Resin	1.22g/ml	WPE=145
	1.15/ml	WPE=160
DM P-30	0.97g/ml	265
BDMA	-1.10g/ml	135

• Cg: Suspected Carcinogen; • C: Corrosive; • F: Flammable; • I: Irritant; • L: Lachrymator; •O: Oxidizing; • T: Toxic; • R: Radioactive; • U: Unlisted as hazard

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The Anhydride: Epoxide Ratio: Quoting freely from Glauerti, industrial applications require that curing conditions are selected so that there is an equivalence of anhydride and epoxide groups resulting in a ratio of anhydride to epoxide (A:E) is 1.0:1.0. Fortunately, the curing temperatures used in electron microscopy during earlier years was set at 60°C or below and, therefore, all possible cross-links were not formed. Full cross-links result in too brittle blocks which are impossible to section. Luft3 reduced the A:E ratio to 0.7:1.1 or 0.7 for embedding media based on Araldite 502 or Eponate 12.

Coulter4 suggested a lower A:E ratio of 0.6 for rapid embedding techniques using a curing temperature of 95°C. Again quoting Glauert: "Under these conditions, the formation of cross-links progresses much faster than at 60°C and a different polymer is most likely to be formed." A-E ratios should only be used when great difficulties are encountered in sectioning. The formula is as follows:

A: E

$$\frac{\text{Volume of anhydride in ml x density of anhydride in g/ml}}{\text{MW of the anhydride}},$$

$$\frac{\text{Volume of epoxy resin in ml x density of epoxy resin in g/ml}}{\text{WIPE of the epoxy resin}}$$

The formula can be used to determine a single mix or the preparation of Luft's A and B mixes for "Epon" embedding. Luft proposed that both mixtures have an A:E ratio of 0.7 (3). The following example should help. Using a generic "Epon" (WPE=145) and with mixtures A and B each containing 25m1 (30.5g) of resin, based on Luft's formulation.

Mix A (DDSA + Resin)

X= $\frac{30.5}{45}$ x 266 x 0.7 X=grams of DDSA required

X=39.2g You need to add 39.2g (39.2m1) of DDSA to 30.5g (25m1) of resin

Mix B (NMA + Resin)

X= $\frac{30.5}{145}$ x 178 x 0.7 X=grams of NMA required

X=26.2 You need to add 26.2g (21.1 ml) of NMA to 30.5g (25m1) of resin

Mixtures A and B are combined in the following proportions to produce blocks of varying hardness.

	ml	ml	ml	ml	ml
Mixture A	10	7	5	3	0
Mixture B	0	3	5	7	10
DMP-30	.2	.2	.2	.2	.2
	Softest	**Soft**	**Medium**	**Hard**	**Hardest**

Hints:

1. Warming 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. Measurements by volume are much easier than measurements by weight.
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.
8. The accelerator BDMA can be dispensed from a graduated pipet or with previous calibrated drops.
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.
10. Volumetric measurements are far simpler than weight measurements.4

1) Glauert AM: Epoxy resins: an update on their selection and use. Microscopy and Analysis, i5-20, Sept. 1991

2) Bils RF.Electron Microscopy Laboratory Manual and Handbook. 2nd ed. Alpha Editions, 106, 1992 (Pella Cat. No. 249-2, page 25, Catalog 9)

3) Luft JH: Improvements in Epoxy Resin Embedding Methods, J Biophys Biochem, Cyto, 9:409-414, 1961

4) Coulter HD Rapid and improved methods for embedding biological tissues in Eponate 12 and Araldite 502. J Ultrastruct Res, 20, 346-345, 1967

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