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Poly/Bed[®] 812 Mini Embedding Media/DMP-30

INTRODUCTION:

Poly/Bed[®] 812 is designed as a replacement for Epon 812. With older formulations of Epon 812 it is more difficult to control the hardness and sectioning quality of the finished blocks. Poly/Bed[®] 812 allows the hardness to be easily controlled without the variability of the WPE (Weight Per Epoxide) calculations required by Luft in previous protocols. Poly/Bed[®] 812 can be directly substituted in most formulations with excellent preservation of the cellular structures required for electron microscopy. Chart 1 on page 2 provides information on WPE.

Poly/Bed[®] allows the tissue sample to be dehydrated with acetone or ethanol, eliminating propylene oxide as an intermediary for infiltration. The loss of phospholipids can be reduced by approximately ten percent by this change in reagents.

LUFT'S FORMULATIONS:

Luft¹⁻⁵ developed a standardized routine formula for embedding with Epon 812, which gave the following advantages over other embedding media^{2,3,4}

1. Faster penetration of the sample and embedding is completed in 24 - 48 hours.
2. Wide range of hardness - varied by changing the ratio of components.
3. Improved contrast - readily stained by osmium tetroxide alone.
4. Easily sectioned in all ranges for Electron Microscopy (EM).
5. Support film not required.

Adding more DDSA for a softer block or more NMA for a harder block to the basic formula can alter block hardness. All mini kit components are mixed at the same time and excess media can be stored in 10cc or 20cc syringes. The syringes should be frozen or stored in a horizontal position. The unused material can be stored at 4°C for 2 weeks or at -20°C for 2 months.

PREPARATION OF POLY/BED[®] 812 STOCK SOLUTION

Poly/Bed [®] 812	50mL in a 120mL mixing bottle
DDSA	30mL
NMA	20mL
DMP-30	5mL

Additional supplies included in the kit:

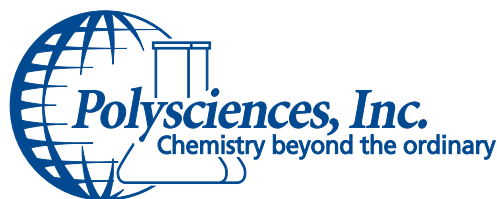
3-10cc Syringes, 1-5cc Syringe, 2-Wooden Stirrers

Add the first three components to the Poly/Bed[®] 812 mixing bottle to make the infiltration and embedding mixture. Stir completely prior to adding the DMP-30. Do not allow air bubbles to form during the mixing process.

The DMP-30 is added as 2mL using a tuberculin syringe. The volume of DMP-30 should be exact.

INFILTRATION:

Fix tissue according to laboratory protocol and dehydrated through a series of ascending ethanol dilutions to absolute alcohol. Dehydration with acetone or ethanol will preserve phospholipids better than propylene oxide. Propylene oxide can be used as an intermediary between the dehydrant and infiltration media, however; it is not required with Poly/Bed[®] 812. Changes should be 5 to 10 minutes depending on tissue size and protocol.



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Several dilutions of stock Poly/Bed[®] 812 and the dehydrant of choice should be used to assist in complete infiltration by the embedding solution. Solutions at 3:1 dehydrant to stock solution should be used with a rotating table or wheel to assure proper infiltration of the specimen. Follow this by a 2:1 mixture, a 1:1 mixture, and finally two changes of the stock solution for the final steps before embedding. Leave tissue in each of these mixtures for a minimum of one hour and overnight for the final step. If a rotator is not available the vials containing the tissue should be mixed several times each hour.

EMBEDDING SAMPLES:

Transfer tissue to the properly labeled embedding capsules or molds preferred by the laboratory. Tissue should be oriented in the bottom of the capsule or mold and the completed embedding solution added. Tissue can be adjusted after the solution is added to assure proper placement. The capsules or molds should be capped if possible.

Polymerize in a 60°C oven for 24 hours. Allow the blocks to cool to room temperature before removing the capsule or mold.

CHART 1 - MIXING POLY/BED[®] 812

WPE is Weight Per Epoxide equivalent. Determine the optimum amount of DDSA or NMA to combine with 100 grams of Poly/Bed[®] 812 resin using this chart for stoichiometric balance. This will minimize un-reacted starting materials and allow control of the block hardness for sectioning. The exact WPE is listed on the label of every lot of araldite resin purchased from Polysciences, Inc.

The hard mix and soft mix are blended according to the desired hardness of a specific specimen or material.

Per 100 Grams of Poly/Bed [®] 812	DDSA Grams for Softer Blocks Resin at WPE	NMA Grams for Harder Blocks
130	112	102
140	104	95
150	97	89
160	91	83
170	85	78

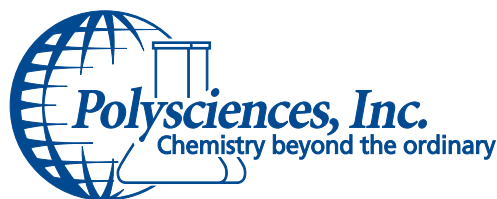
CAUTION:

The toxicological properties of Poly/Bed[®] 812 are not fully known. Prolonged repeated contact of liquid or breathing vapors or mists may cause delayed and serious injury. Do not get on skin, clothing or in eyes. Avoid inhalation of vapors and mists. In case of contact with eyes or skin, immediately flush with copious amounts of water for at least 15 minutes; for eyes get immediate medical attention. Remove contaminated clothing and shoes at once. Clothing should be destroyed. 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.

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ORDERING INFORMATION:

Cat. #	Description	Size
08791	Poly/Bed® 812	500g
08792	Poly/Bed® 812 (Luft's Formulation) Embedding Kit	1 kit
21958	Poly/Bed® 812 - DMP -30 Mini Kit (Luft's Formulation)	1 kit
21844	Poly/Bed® 812 Embedding Kit - BDMA (Glauert Formulation)	1 kit
21959	Poly/Bed® 812 - BDMA Mini Embedding Kit (Glauert Formulation)	1 kit
02595	Araldite Embedding Kit, Mollenhauer	1 kit
00553	DMP-30	100g
00563	Dodecensylsuccinic anhydride (DDSA)	450g, 4 x 450g
00886	Nadic methyl anhydride (NMA)	450g
00236	Propylene oxide, EM Grade	1 pint
09860	Reagent Grade Alcohol (100%)	1 gallon
00224	BEEM* Capsules Size 00	pkg of 100 / 500 / 1000
00294	BEEM* Capsules Size 00 Conical	pkg of 100
00336	BEEM* Capsules Size 3	pkg of 100
00295	BEEM* Capsules Size 00 Bottle Neck	pkg of 100
00225	Gelatin, Embedding capsules Size 00 (23.3mm L x 8.18mm W x 0.95mL volume)	pkg of 1000
07347	Gelatin, Embedding capsules Size 1 (19.0mm L x 6.63mm W x .50mL volume)	pkg of 1000
07348	Gelatin, Embedding capsules Size 3 (13.9mm L x 5.05mm W 0.21mL volume)	pkg of 1000
23257	BEEM®, Flat Transparent	1 mold
19440	Chien Universal Mold	1 mold
0256A	BEEM® Capsule Holders for Embedding	3 holders, 12 holders
0256B	BEEM® Size 3 Capsule Holders for Embedding	3 holders, 12 holders
08408	Capsule, Micron Micromolds (50 units of 10 capsules)	1 pkg
00631	Formvar 15/95 (Poly[vinyl formal])	100g
04672	Formvar in 0.5% solution in ethylene dichloride	100g
00785	Lead (II) Nitrate, 99% for Reynolds Lead Citrate Solution	100g
21447	Uranyl Acetate 98%, ACS Grade	25g

*BEEM is a registered trademark of Better Equipment for Electron Microscopy, Inc.

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