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TECHNICAL DATA SHEET 233

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Poly/Bed[®] 812 Embedding Media/DMP-30 Kit Catalog # 08792-1

Poly/Bed[®] 812 Embedding Media Catalog # 08791-500

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 3 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. This change of reagents reduces the loss of phospholipids by approximately ten percent.

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 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.

Chart 1 provides Luft's formulas for adjusting the hardness of Poly/Bed[®] 812. Altering the formula by adding more DDSA will result in a softer block. Adding more NMA results in a harder block. The formula can be made up in 5X quantities and stored for future use. This allows more control of the infiltration and embedding solution over time. It will also limit exposure to hazardous components.

Preparation of Poly/Bed[®] 812 Stock Solution (100ml)

Poly/Bed [®] 812	51.13g or 48mL
DDSA	27.02g or 31mL
NMA	21.85g or 21mL
DMP-30*	2.00g or 2mL (Added just prior to use.)

*Add DMP-30 just prior to infiltration and embedding. Use a tuberculin syringe to exactly measure the volume of DMP-30. If the quantities given in are divided into equal aliquots of 25mL each, 0.5mL of DMP-30 should be added for the final solution.

Use the stock solution immediately or store at -20°C in 10cc or 20cc syringes. Freeze the syringes in a horizontal position. Allow them to thaw completely, at least overnight in a cool, dark area. The quantities indicated can be measured by either volume or weight. Measure or weigh all components under a hood. The solution should be mixed well prior to adding DMP-30.

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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.

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 of Poly/Bed® 812 for the final steps before embedding. The tissue should be in each of these mixtures for a minimum of one hour and the final step can be overnight. If a rotator is not available the vials containing the tissue should be mixed several times each hour.

Embedding Samples:

The amount of stock solution required for the infiltration/embedding procedure should be prepared by adding the amount of DMP-30 required for final polymerization.

1. Combine 25mL of stock solution with 0.5mL of DMP-30 and mix well. This should be prepared immediately prior to use as the DMP-30 can cause the solution to thicken.
2. Transfer tissue to a properly labeled embedding capsules or mold preferred by the laboratory. Tissue should be oriented in the bottom of the capsule or mold and the 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.
3. Polymerize in a 60°C oven for 24 hours. Allow the blocks to cool to room temperature before removing the capsule or mold.

Poly/Bed® 812 - Araldite Mixture:

Mollenhauer⁶ has used Epon-Araldite mixtures to produce blocks with high image contrast. The blocks were easier to section than those embedded in either Epon 812 or Araldite alone. Poly/Bed 812 can be substituted in this formula with excellent results. Measure all components under a hood.

Standard Mollenhauer Araldite Mixture:

Poly/Bed® 812	100.0ml
Araldite 502	60.0ml
DDSA	18.0ml
DMP-30	5.2ml (Added just prior to use)

Add DMP-30 just prior to use in the amount required for the volume used to infiltrate and embed. A 44.50ml volume of stock solution would require 1.30ml of DMP-30.

This mixture can be stored in 10cc to 20cc syringes at -20°C for up to two month or at 4°C in an airtight syringe for two weeks. The syringes should be in a horizontal position to freeze or store.

Dehydration and infiltration should be completed as directed in the standard Poly/Bed® 812 directions.

Variation of Mollenhauer Araldite Mixture:

Poly/Bed® 812	10.0 ml
Araldite 502	10.0 ml
DDSA	24.0 ml
DMP-30	0.9 ml (Added just prior to use)

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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.

References:

1. Kushida, H., Electron Micro., **8**, 72, (1959)
2. Finck, H.J., Biophys. and Biochem. Cytol. **7**, 27 (1960)
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4. Glauert, A.M., et al., Nature, **178**, 803, (1956)
5. Luft, J.J., Biophys. Biochem. Cytol., **9**, 409, (1961)
6. Mollenhauer, H.H., Stain Technology, **39**, 11, (1964)
7. Juengel, L.I., Proceed. 43rd EMSA, 446, (1985)
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Ordering Information:

Cat. #	Description	Size
08791-500	Poly/Bed® 812	500gm
087929-1	Poly/Bed® 812 (Luft's Formulation) Embedding Kit	kit
21958-1	Poly/Bed® 812 (Luft's Formulation) Mini Embedding Kit	kit
21844-1	Poly/Bed® 812 Embedding Kit/ - BDMA (Glauert Formulation)	kit
21959-1	Poly/Bed® 812/BDMA Mini Embedding Kit (Glauert Formulation)	kit
02595-1	Poly/Bed® Araldite 502 Embedding Kit	kit
02960-1	Poly/Bed® Araldite 502 Mini Embedding Kit	kit
00553-100	DMP-30	100g
00563-450	Dodecensylsuccinic anhydride (DDSA)	450g
00563-4	Dodecensylsuccinic anhydride (DDSA)	4 x 450g

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00886-450	Nadic methyl anhydride (NMA)	450g
00236-1	Propylene oxide, EM Grade	1pt.
00236-6	Propylene oxide, EM Grade	6 x 1pt.
01914-4	Acetone, EM Grade 99.5%	4 x 1L
09860-1	Reagent Grade Alcohol (100%)	1 gallon
00224-100	BEEM* Capsules Size 00	1pkg of 100
00294-100	BEEM* Capsules Size 00 Conical	1pkg of 100
00336-100	BEEM* Capsules Size 3	1pkg of 100
00295-100	BEEM* Capsules Size 00 Bottle Neck	1pkg of 100
00225-1	Gelatin, Embedding capsules Size 00 (23.3mm L x 8.18mm W x 0.95mL volume)	1pkg of 1000
07347-1	Gelatin, Embedding capsules Size 1 (19.0mm L x 6.63mm W x .50mL volume)	1pkg of 1000
7348-1	Gelatin, Embedding capsules Size 3 (13.9mm L x 5.05mm W 0.21mL volume)	1pkg of 1000
23257-1	BEEM®, Flat Transparent	1 mold
23256-1	Chien 8 Faced Mold	1 mold
19440-1	Chien Universal Mold	1 mold
0256A-3	BEEM® Capsule Holders for Embedding	3 holders
0256A-12	BEEM® Capsule Holders for Embedding	12 holders
0256B-3	BEEM® Size 3 Capsule Holders for Embedding	3 holders
0256B-12	BEEM® Size 3 Capsule Holders for Embedding	12 holders
08408-1	Capsule, Micron Micromolds (50 units of 10 capsules)	1 pkg
00631-100	Formvar 15/95 (Poly[vinyl formal])	100g
04672-100	Formvar in 0.5% solution in ethylene dichloride	100g
00785-100	Lead (II) Nitrate, 99% for Reynolds Lead Citrate Solution	100g
21447-25	Uranyl Acetate 98%, ACS Grade	25g

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