

Poly/Bed[®] 812 - BDMA Embedding Kit

(A.M. Glauert Method) Catalog Number 21844-1

Introduction

Epoxy resins were introduced as embedding media for Electron Microscopy in 1961¹ and they are still widely used today. The classic formulation for Epon 812 by Luft¹ introduced in 1961¹, has been published in many variations for use with biological and material science samples for electron microscopy. Poly/Bed[®] 812 is the exact equivalent of Epon 812, which is no longer commercially available. In the original formulation Luft¹ recommended the use of DMP-30 as an accelerator for polymerization. The alternative suggested by A.M. Glauert²⁻³ is BDMA (benzyl dimethyl amine). The BDMA mixture offers a lower viscosity media for faster penetration of the sample. The lower viscosity allows this mixture to be used with denser materials not well penetrated by Poly/Bed[®] 812/ DMP-30.

Poly/Bed[®] 812 Embedding Media/BDMA Kit 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.

Preparation of Stock Poly/Bed[®] 812 Embedding Media/BDMA Solution

This is a medium hard embedding media. It may not be appropriate for very hard samples. The components are pre-measured for ease of handling and less exposure to the material.

Gloves should be worn at all times while handling the components for mixing, infiltration, and embedding.

<u>Kit Component</u>	<u>Description</u>	<u>Quantity</u>
Component A	Poly/Bed [®] 812	200mL (in a 500mL bottle)
Component B	DDSA (Dodecenylsuccinic anhydride)	160mL
Component C	NMA (Nadic methyl anhydride)	100mL
Component D	BDMA* (Benzyl dimethyl amine)	20mL

* BDMA is very sensitive to humidity and should be tightly capped at all times.

1. Carefully pour Component B and Component C in the 500mL bottle containing Component A.
2. Mix gently. Do Not Allow Air Bubbles to Form! Mixing can be completed on a rotator or with a magnetic stir plate agitating gently.
3. Only 14mL of Component D are required to complete the mixture. Carefully measure 14mL of BDMA with a syringe or other accurate system and add to the 500mL bottle.
4. Gently mix all the ingredients. Air bubbles may form during mixing. Air bubbles may be removed by loosely covering the bottle top with Parafilm and placing in a vacuum chamber. Be sure to punch holes in the Parafilm to allow degassing of the mixture. Alternatively, air bubbles can be removed with very gentle heat at approximately 40°C for a brief period. This will also aid in infiltration of the mixture for difficult samples. Do not over heat as the sample could begin to polymerize with extended exposure. Do not exceed one hour.

Storage of Stock Solution:

The mixture can be stored in capped 10cc or 20cc syringes at -20°C for two months. The syringes should be frozen in a horizontal position. The frozen mixture should be allowed to unthaw completely prior to use.

If the mixture is not completely used for infiltration and embedding it should be allowed to polymerize before disposal. Polymerize at 60°C for 24 to 48 hours.

Infiltration:

Tissue is fixed 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/BDMA 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. This followed by a 2:1 mixture a 1:1 mixture and finally two changes of the stock solution 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:

Tissue should be transferred 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 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.

Polymerization should be complete after 24 to 48 hours in a 60°C oven. Allow the blocks to cool to room temperature before removing the capsule or mold.

Caution:

The toxicological properties of Poly/Bed® 812 are not fully known. Gloves should be worn at all times while handling the components of this kit. 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. Luft, J.J. Biophys. Biochem. Cytol., 9:409 (1961)
2. Glauert, A.M., et al., Nature, 178:803 (1956)
3. Glauert, A.M.
4. Zacks, S. I., Stain Technology, 38

Ordering Information:

Cat #	Description	Size
08791-500	Poly/Bed® 812	500gm
08792-1	Poly/Bed® 812 Embedding Kit (Luft's Formulation)	kit
21958-1	Poly/Bed® 812 - DMP-30 Mini Kit (Luft's Formulation)	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
00886-450	Nadic methyl anhydride (NMA)	450g
00236-1	Propylene oxide, EM Grade	1 pint
00236-6	Propylene oxide, EM Grade	6 x 1 pint
01914-4	Acetone, EM Grade, 99.5%	4 x 1L
01914-6	Acetone, EM Grade, 99.5%	6 pints
09860-1	Reagent Grade Alcohol (100%)	1 gallon
00224-100	BEEM® Capsules Size 00	100/pkg
00294-100	BEEM® Capsules Size 00 Conical	100/pkg

00336-100	BEEM® Capsules Size 3	100/pkg
00295-100	BEEM® Capsules Size 00 Bottle Neck	100/pkg
00225-1	Gelatin, Embedding Capsules Size 00 (23.3mm L x 8.18mm W x 0.95mL volume)	1000/pkg
07347-1	Gelatin, Embedding Capsules Size 1 (19.0mm L x 6.63mm W x .50mL volume)	1000/pkg
07348-1	Gelatin, Embedding Capsules Size 3 (13.9mm L x 5.05mm W 0.21mL volume)	1000/pkg
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
0256B-3	BEEM® Size 3 Capsule Holders for Embedding	3 holders
08408-1	Capsule, Micron Micromolds	50 units/pkg
00631-100	Formvar 15/95 (Poly[vinyl formal])	100g
04672-100	Formvar in 0.5% ethylene dichloride solution	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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