U.S. Corporate Headquarters
400 Valley Road
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
Fax: 1(800)343-3291
Email: info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3 D-69214
Eppelheim, Germany
+(49) 6221-765767
Fax: +(49) 6221-764620
Email: info@polysciences.de

EMBEDDING GUIDE FOR EPON® RESINS

EMBEDDING MEDIA

Ideal qualities of embedding medium:

1. Easily available
2. Uniformity from one batch to another, no lot to lot variation
3. Solubility in dehydrating agents
4. Low viscosity as monomer for penetration
5. Uniform polymerization
6. Little volume change during polymerization
7. Good preservation of fine structure
8. Good sectioning quality that includes homogeneity, hardness, plasticity and elasticity
9. Resistance to heat generated by sectioning
10. Adequate specimen stainability
11. Stability in electron beam
12. Electron lucent

Epoxy resins, polyester resins and methacrylates are in general use. For general electron microscopy epoxy resins have most properties required.

EPOXY RESINS

ADVANTAGES

1. Polymerize uniformly with little change in volume (as low as 2%).
2. Relatively stable in electron beam.
3. Mixtures can be stored for several weeks at 4˚ C and many months at -20˚ C in well sealed containers.

DISADVANTAGES

1. Relatively high viscosity, which necessitates a lengthy graded infiltration procedure.
2. Some reduction in contract between specimen and background.
3. Can cause severe irritation to skin.

CHARACTERISTICS

1. Polyaryl ethers of glycerol with terminal epoxy groups.
2. Transparent yellowish resins which range from viscous liquids to fusible solids.
3. Require addition of curing agents to convert them to a tough, extremely adhesive and highly inert solid. Polymerization accomplished by the addition of various bifunctional setting groups which link with the resin to produce a three-dimensional structure.

EMBEDDING MIXTURE CONTAINS

1. Epoxy resin
2. Plasticizer and/or flexibilizer
3. Hardener
4. Accelerator (catalyst)

Plasticizer does not become part of cross-linked structure. It is necessary in some mixtures to prevent block from becoming too brittle and to improve sectioning properties.

Flexibilizer reacts with epoxy resins and becomes part of cross-linked structure. They are less likely to be lost under electron beam than plasticizers.

Hardener: Nature influences hardness of block.

Plasticizers, flexibilizers and hardeners influence hardness of blocks. Proportions may be varied to alter hardness. Accelerator also influences hardness but variation in concentration may cause blocks to become brittle and difficult to section. Sectioning properties depend on hardness and extent and nature of cross-links formed during polymerization.

ARALDITE RESINS

CHARACTERISTICS

Glycerol based aromatic resin that has very little volume shrinkage on polymerization.

High viscosity:
Araldite 502 = 3000 cps at 25˚ C
Araldite CY 212 = 1300 to 1650 cps at 25˚ C

Rather low softening temperature.

Not reactive with alcohols. Transitional solvent (acetone or propylene oxide) necessary between dehydration alcohols and embedding.

Sections have excellent stability under electron beam and stain well.

U.S. Corporate Headquarters
400 Valley Road
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
Fax: 1(800)343-3291
Email: info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3 D-69214
Eppelheim, Germany
+(49) 6221-765767
Fax: +(49) 6221-764620
Email: info@polysciences.de

EXAMPLE OF EMBEDDING MIXTURE (LUFT, 1961)

Araldite 502	27 ml
DDSA (hardener)	23 ml
DMP-30 (accelerator)	0.75 to 1 ml

Mix thoroughly!

Araldite 502 contains plasticizer as supplied by the manufacturer. Final hardness may be varied by changing the resin/hardener ratio.

Polymerize at 45˚ C for 36 hr or 60-70˚ C overnight.

EPON

Epon 812 has been the most widely used embedding resin. The manufacturer (Shell) has discontinued production. Substitutes, such as Poly-Bed 812 have been developed by Polysciences, Inc.

CHARACTERISTICS

Glycerol based aliphatic epoxy resin.

Relatively low viscosity: Epon 812 = 150-220 cps at 25˚ C.

Easily hardened at low temperatures.

Not reactive with alcohol. Requires use of transition solvent (acetone or propylene oxide).

Cutting quality influenced by:

1. hardener/epoxy ratio
2. final block hardness
3. temperature and duration of polymerization

Sections show greater contrast in electron microscope than araldite sections. Epon has inherent granularity which may limit high magnification and high resolution studies.

EXAMPLE OF EMBEDDING MIXTURE (LUFT, 1961)

Mixture A

Epon 812	62 ml
DDSA (hardener	100 ml

Mixture B

Epon 812	100 ml
NMA (hardener)	89 ml

Combine mixture A with mixture B. Add accelerator (BDMA or DMP-30) in proportion of 1.5-2%.

	ml	ml	ml	ml	ml
Mixture A	10	7	5	3	0
Mixture B	0	3	5	7	10
BDMA or DMP-30	0.15	0.15	0.15	0.15	0.15

Soft----------> Hard

Mix components thoroughly!

Polymerize at 45˚ C for 12 hr and 60˚ C for 24 hr at 60-70˚ C overnight.

Mixtures A and B can be stored in refrigerator for several months. Epon is hydroscopic so storage bottles should be well sealed.

NMA reacts with permanganates. Mixtures with NMA cannot be used in conjunction with permanaganates.

EPON-ARALDITE COMBINATIONS (MOLLENHAUER, 1964)

Mixture	#1	#2	#3
Epon 812	25 ml	62 ml	-
Araldite 502 or 6005 (or CY 212 in Europe)	15 ml	-	-
Araldite 506	-	81 ml	50 ml
DDSA (hardener)	55 ml	100 ml	-
Cardolite NC-513 (flexibilizer)	-	-	25 ml
DBP (plasticizer)	2-4 ml	4-7 ml	1-2 ml
DMP-30	1.5%	1.5%	-
or BDMA	3%	3%	3%
Relative hardness	medium	soft-medium	soft-medium
Image contrast	high	medium	low
Tissue preservation	good	excellent	excellent

U.S. Corporate Headquarters
400 Valley Road
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
Fax: 1(800)343-3291
Email: info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3 D-69214
Eppelheim, Germany
+(49) 6221-765767
Fax: +(49) 6221-764620
Email: info@polysciences.de

NOTES

Developed for plant tissues but applicable for wide range of specimens with little modification.

Mixture #1 is easier to section than media containing epon or araldite alone.

Mixture #2 is easier to section than mixture #1.

Mixture #3 is slightly more difficult to section than mixtures 1 & 2 and image contrast is lower. Preservation of specimen is excellent and mixture is particularly useful for specimens (ex. pollen grains) which tend to be pulled out of the block during sectioning.

BDMA should replace DMP-30 in mixture #3 since DMP-30 causes precipitate formation during infiltration.

ERL (VINYL CYCLOHEXENE DIOXIDE) AKA SPURR'S RESIN

CHARACTERISTICS

Cycloaliphatic diepoxide.
Lowest viscosity of resins: 7.8 cps at 25˚ C. Facilitates rapid penetration into specimen.
Miscible with ethanol. Transition solvent can be eliminated
Resistant to electron beam.
Resistant to oxidation by permanganates.

EXAMPLE OF EMBEDDING MIXTURE

Spurr's low-viscosity embedding resin (Spurr, 1969)

All components have low viscosity.

ERL 4206	7.8 cps at 25˚ C
DER 736	30-60 cps at 25˚ C
NSA	117 cps at 25˚ C

Viscosity of final medium is 60 cps at 25˚ C.

SPURR RESIN MIXTURES

Mixture	A	B	C	D	E	F
	gm	gm	gm	gm	gm	gm
ERL 4206	10	10	10	10	10	10
DER 736 (flexibilizer)	6	5	4	8	6	6
NSA (hardener)	26	26	26	26	26	26
DMAE (S1, accelerator)	0.4	0.4	0.4	0.4	1	0.2
Hardness	Firm	F/Hard	Hard	Soft	Firm	Firm
Pot life (days)	3-4	3-4	3-4	3-4	2	7
Polymerization time (hrs)	8	8	8	8	3	16

NOTES

Mixture A is the standard mixture. Mixtures B-F are modifications.
Mixture E has more accelerator. Time required for polymerization is reduced by storage (pot life) time is shortened.
Mixture F has reduced accelerator. Time required for polymerization is increased, but mixture can be stored in refrigerator for over 2 weeks.
Note: 0.4 gm accelerator = 0.5 ml measured in a plastic disposable syringe.

ORDERING INFORMATION:

CAT #	DESCRIPTION
02334	Epon® Resin 828
08792	Poly/Bed® 812 (Luft formulations) Embedding Kit / DMP-30
21960	Poly/Bed® Araldite 502 Mini Kit
21959	Poly/Bed® 812 Mini Kit/BDMA (Glauert Version)
21844	Poly/Bed® 812 Embedding Kit /BDMA (Glauert Version)
08791	Poly/Bed® 812 Embedding Media

TO ORDER

In The U.S. Call: **1(800) 523-2575 • (215) 343-6484**
In The U.S. Fax: **1(800) 343-3291 • (215) 343-0214**

In Germany Call: **+(49) 6221-765767**
In Germany Fax: **+(49) 6221-764620**

Order online anytime at **www.polysciences.com**