

Laboratory for Advanced Electron and Light Optical Methods

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Processing Procedures for JB-4 Resin

A. Fixation

Fix samples with McDowell's and Trump's 4F:1G fixative. Do not use osmium (or Bouin's fixative; the picric acid will soften the plastic).

B. Rinse 2X (10 min each) in Sorenson's Sodium Phosphate Buffer (pH 7.2-7.4, 0.1M).

C. Dehydration

50% ethanol 15 min

75% ethanol 15 min

95% ethanol 15 min (X2)

100% ethanol 30 min (X2)

D. Infiltration

1. Mix up one batch of catalyzed infiltration resin:

100 mL of JB-4 Solution A

Add 0.90 g of dry catalyst C; mix until dissolved.

It helps to place the dry catalyst into a weighing dish and to mix it with a small amount of Solution A to form a paste before adding the rest of Solution A, putting the solution in a Nalgene bottle and mixing with a stirring bar for 30-60 min. Make sure that the catalyst is in solution before proceeding.

Note: The solution may be stored for several weeks at approximately 4 °C in the dark.

2. Infiltration: put samples (2 mm³ blocks maximum) in infiltration medium for about 5 hrs at room temperature on rotator. Change to new medium after about 2.5 hrs.

D. Embedding

1. Mix up one batch of embedding medium:

1 ml of JB-4 Solution B

25 ml of fresh catalyzed Solution A.

Note: Never use exhausted infiltration medium for embedding.

Stir mixture thoroughly and place into ice bath while arranging samples in molds to prevent premature polymerization.

2. Place samples in embedding molds.

Place samples in BEEM capsules and fill molds with embedding medium. Molds must be filled entirely with embedding medium as air will prevent proper polymerization. Cap samples and place samples in a desiccator overnight at room temperature over a bed of Drierite. The Drierite will help produce blocks of good hardness, without sticky surfaces.

E. Sectioning

1. Trim off excess plastic with a razor blade but be sure to leave some excess around all the edges because they will curl slightly.
2. Break triangular glass knife, orient at 10° and cut 2-3 μm thick sections.
3. Pick up dry sections with forceps and release onto surface of water in staining dish (adding a drop of ammonium hydroxide may help the sections spread).
4. Pick up several sections on glass slides and heat on hot plate at approximately 70°C for about 15 min.

F. Staining

Stain with appropriate stains per individual protocol.

G. Coverslip

Dry on a hotplate at approximately 60°C and add drop of Polymount. Place #1.5 coverslip over sections and examine.