PREPARATION OF SPI-Pon 812 with BDMA EMBEDDING MEDIA

This is a medium hard embedding media. It may not be appropriate for very hard samples.
The SPI-Pon812/BDMA is an alternative to the original formulation introduced by Luft (1) in 1961.
The SPI-Pon 812/BDMA version is the one developed by A.M.Glauert (2-3).
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

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

Kit Component Description and Volume (Quantity) :

SPI-Pon 812 - 450mL bottle
DDSA (Dodecenylsuccinic annhydride) – 450ml bottle
NMA (Nadic methyl annhydride) – 450ml bottle
BDMA* (Benzyl dimethyl amine) – 2 x 30ml bottles
* BDMA is very sensitive to humidity and should be tightly capped at all times.

1. Carefully pour 160ml of DDSA + 100ml of NMA into 200ml of SPI-Pon 812: total = 460ml
2. Mix gently. *Do Not Allow Air Bubbles to Form!* Mixing can be completed on a rotator or with a magnetic stir plate by agitating gently.
3. Carefully measure 14mL of BDMA with a syringe or other accurate system and add to the above mixture
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.

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.

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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 SPI-Pon 812.

Changes should be 5 to 10 minutes depending on tissue size and protocol.
Several dilutions of the SPI-Pon 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. (BEEM capsules, SPI# 02305, 02310, 02320 or 02330 are suggested)

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.

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.

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