

Tissue Processing Dehydration to Plastic

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Abstract

Tissue preparation in the electron microscopy lab to prepare the EMbed 812 for tissue processing.

§1 Introduction

This document is intended to be used to process tissue from formalin to embedded plastic to be used on the transmission electron microscope (TEM) to identify the orientation of collagen fibers.

§2 Embed 812

§2.1 Personal Protective Equipment

Begin first by grabbing a lab coat and then use paper towels and acetone to clean off the scale used for measuring out the mass of various resin mixtures.

§2.2 Recipe for EMbed 812

Remove the four chemicals for the EMbed 812 resin from the cabinet by using the WPE-147. Where W.P.E. is the Weight per Epoxide Equivalent).

Ingredient	Unit
EMbed 812 Resin	51.80 g
DDSA	26.68 g
NMA	21.67 g
BDMA	2.5 ml

Table 1: Simplified instructions to check and make sure the automatic tissue processor is set up at the correct stations. Each vial should be filled with 20 ml when processing. Be sure to check the program on the automatic tissue processor; it should be marked by program #2.

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§3 Lab Equipment

Grab one (400 ml) Tripore container along with four clean pipettes.

§4 EMBED 812

Balance the scale with the Tripore container and pour in 51.80 g of EMBED 812 Resin. Clean the bottle and cap with Kimwipes and throw out the pipette. Grab a strip of Parafilm to stretch over the cap to ensure an air-tight seal.

§5 DDSA

Balance the scale after 51.80 g of EMBED 812 Resin has been added to the Tripore container. Add 26.68 g of DDSA to the container by first underpouring and then using a pipette to add the rest of the DDSA. Clean the bottle and cap with Kimwipes and throw out the pipette. Grab a strip of Parafilm to stretch over the cap to ensure an air-tight seal.

§6 NMA

Balance the scale after 26.68 g of DDSA has been added to the Tripore container. Add 21.67 g of NMA to the container by first underpouring and then using a pipette to add the rest of the NMA. Clean the bottle and cap with Kimwipes and throw out the pipette. Grab a strip of Parafilm to stretch over the cap to ensure an air-tight seal.

§7 Stir

Move the Tripore container inside the hood. Add a stirbar to the Tripore container containing EMBED 812 Resin, DDSA, and NMA to stir the mixture for 10 minutes in the hood.

§8 BDMA

In the hood, while the Tripore container is being stirred, use a graduated micropipette and obtain 2.5 ml of BDMA. The BDMA is used as the accelerant for polymerization.

§9 Stir

Stir the Tripore container containing EMBED 812 Resin, DDSA, NMA, and BDMA mixture for 10 minutes in the hood. The mixture should turn orange after the BDMA has been added.

§10 Parafilm

Using Parafilm wrap, ensure that each chemical lid has been wrapped to keep an air-tight seal.

§11 Syringe

Grab eight syringes and caps from the cupboards and prepare them for filling up with the resin. Place the newly filled resin syringes in the freezer.

§12 Clean-up

Clean the magnetic stirbars with acetone. Be sure to put vinyl liners inside the gloves.

§13 Embed

Embed the tissue samples!