

Preparation of Costar® Transwell® Inserts for Transmission Electron Microscopy



Supplies

Reagents

Hank's balanced salt solution (HBSS)
Glutaraldehyde, 70% E.M. (Polysciences Cat. # 01201)
Osmium tetroxide, crystalline (Polysciences Cat. # 0223A)
Sodium cacodylate (Polysciences Cat. # 01131)
Sucrose, reagent grade
Calcium chloride
Lead nitrate (Polysciences Cat. # 00785)
Sodium citrate (Polysciences Cat. # 02121)
Sodium hydroxide
Uranyl acetate (Polysciences Cat. # 00379)
Ethanol 100% and 95%
Toluidine blue stain (0.05%) (Polysciences Cat. # 1234)
LR White medium grade (Ted Pella, Inc. Cat. # 18181; hard grade Cat. # 18182)

Materials

Flat embedding molds (Polysciences Cat. # 02615)
Fine forceps
Teflon spray (Polysciences Cat. # 03928)

Protocol

WARNING: Appropriate safety precautions must be used when handling fixative solutions and the highly reactive osmium tetroxide, which gives off a reactive vapor.

1. Remove medium and rinse cells with Hank's balanced salt solution prewarmed to 37°C. This should be a gentle rinse, letting the liquid flow down the sides of the Transwell insert.
2. Fix monolayers with 2.5 -3.0% glutaraldehyde in cacodylate buffer (0.1 M), pH 7.2 -7.4 for 1 hour at room temperature. **Wear gloves and protective eye goggles.**
3. Rinse twice with cacodylate buffer adjusted to 300milli-osmols with 6.84% sucrose.
4. Postfix with 1% osmium tetroxide in cacodylate buffer (0.1 M), pH 7.2 -7.4 for 1 hour at 4°C. **Use latex gloves, work in a fume hood and have a proper disposal bottle available for the used osmium.**
5. Rinse twice with cacodylate-sucrose buffer.
6. Dehydrate through a graded series of ethanol concentrations:
 - 35% ethanol for 10 minutes
 - 50% ethanol for 10 minutes
 - 70% ethanol for 10 minutes
 - 95% ethanol for 10 minutes
 - 100% ethanol for 10 minutes
 - 100% ethanol for 10 minutesAcetone should not be used as remaining traces of it can interfere with polymerization.
7. Infiltrate with complete embedding resin (LR white, medium or hard grade) as follows:
 - 75% ethanol: 25% resin for 30 minutes
 - 50% ethanol: 50% resin for 30 minutes
 - 25% ethanol: 75% resin for 30 minutesThe above is to replace intermediate solvents such as propylene oxide.
8. Complete resin hardening overnight at room temperature (16 hours).
9. At a time point 2 days prior to fixation and infiltration of the monolayer, complete resin is poured into an aluminum dish to a depth of 2 -3 mm and allowed to polymerize in a 60°C oven.
10. The membrane, while still in the Transwell insert, is removed from the multiple well plate and the resin is poured off. The membrane and Transwell insert are placed on top of the hardened resin and fresh complete resin is added to cover the membrane. The preparation is placed in a 60°C oven and allowed to polymerize for 72 hours. (This approach allows the membrane to be embedded without removing it from the insert. It is difficult to handle an isolated membrane, an even though it can be cut out of the insert after fixation, it tends to fold and roll up; both of which will produce artifacts.)

11. The aluminum dish is removed and the area of the resin containing the membrane is cut away from the excess material using a fine jeweler's saw. The blocks are trimmed. Thick and thin sections can now be prepared.

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