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Preparation of Falcon® Cell Culture Permeable Supports for Scanning Electron Microscopy

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Introduction

Falcon Cell Culture Permeable Supports (Cat. Nos. 353090 and 353095) feature smooth, transparent, microporous membranes that provide an excellent substrate for cell culture. For most histological procedures, the culture permeable supports can be processed intact, using standard techniques, by passing them through a series of fixation and staining solutions. The membrane, which offers excellent chemical resistance to organic solvents, can be easily cut with a razor blade or scalpel in order to remove samples for embedding, sectioning or staining. Falcon Cell Culture Permeable Supports can be highly recommended for both transmission and scanning electron microscopy (SEM) procedures. We recommend the following procedure for the preparation of Falcon Cell Culture Membrane Permeable Supports for SEM.

Materials and Methods

- Dulbecco's phosphate buffered saline (PBS, with calcium and magnesium salts)
- Sodium Cacodylate (e.g., Cat. No. 1131, Polysciences, Inc.)
- Glutaraldehyde (e.g., Cat. No. 18428,50%, E.M. Grade, Polysciences, Inc.)
- Osmium tetroxide (e.g., Cat. No. 972B, 4% aqueous osmic acid, Polysciences, Inc.)
- Reagent Grade Sucrose
- Absolute ethanol (and 95% ethanol)
- Hexamethyldisilazane (HMDS, Cat. No. 0692, Polysciences, Inc.)
- Colloidal Silver ("silver-dag", Cat. No. 3208, Polysciences, Inc.)

Fixation Procedure

Steps 1-4 are done at 4°C, on ice, or in refrigerator.

1. Aspirate medium and gently rinse permeable support with Dulbecco's phosphate buffered saline (PBS + CA^{++}, Mg^{++}, pH 7.4). Add PBS to both permeable support and well and allow to stand for one to two minutes.
2. In fume hood, replace PBS with primary glutaraldehyde fixative: 0.1 M sodium cacodylate, 5% glutaraldehyde, 0.1 M sucrose, pH 7.4. Add fixative to both permeable support and well and allow fixation to proceed for 30-45 minutes.
3. Remove glutaraldehyde fixative and replace it with buffered sucrose vehicle: 0.1 M sodium cacodylate, 0.1 M sucrose, pH 7.4. Add buffered sucrose to permeable support and well and allow to stand for 30 minutes.
4. Replace buffered sucrose (in both permeable support and well) with osmium fixative: 0.1 M sodium cacodylate, 0.1 M sucrose, 1% osmium tetroxide, pH 7.4. Allow secondary osmium fixation to proceed for 60 minutes. If necessary, permeable support may be rinsed at this point with cold PBS and stored in PBS at 4°C for an indefinite length of time following secondary fixation. If permeable supports are to be stored in this manner, change the PBS several times during the first one to two days of storage to remove unreacted osmium.

Dehydration and Drying Procedure

Steps 5-8 are done at room temperature.

5. Following fixation at 4°C (and possibly storage in PBS as described), allow the permeable support to come to room temperature and wash for five minutes in distilled water; repeat the five-minute wash with distilled water. Do not allow the membrane to partially air-dry at any stage of the following ethanol dehydration procedure.
6. Dehydrate the intact permeable support by sequentially immersing it in the following ethanol solutions made up with distilled water (fill both well and permeable support, as usual).
   - 35% ethanol in H_2O for 10 mins.
   - 70% ethanol in H_2O for 10 mins.
   - 85% ethanol in H_2O for 10 mins.
   - 95% ethanol in H_2O for 10 mins.
   - 100% ethanol (absolute) for 20 mins.
7. If desired, the dehydrated permeable support may then be dried in a critical point drying (CPD) apparatus using standard techniques. Alternatively, we have achieved excellent results by air-drying permeable supports from a solution of hexamethyldisilazane (HMDS, Polysciences, Inc.)[2,3,4] This rapid, simple and inexpensive procedure requires no special CPD apparatus. If HMDS is to be used, simply transfer the permeable support from 100% ethanol to HMDS (100%) for five minutes (dispense liquid into both wells and permeable support, in a fume hood; HMDS is toxic).
8. Remove permeable support from HMDS and air dry at room temperature for 30 minutes. If membrane samples cannot be cut and mounted immediately, the permeable support should be stored with desiccant or in a vacuum desiccator.

continued

Mounting and Coating Procedure

Steps 9-11 are done at room temperature.

9. Remove membrane (with the fixed and dried cells) from the base of the permeable support using a scalpel or razor blade. Cut into small individual specimens.
10. Mount membrane samples on SEM stubs using conducting colloidal graphite or silver suspension ("silver-dag") to make several low resistance contact points between the stub and the sample. Ensure good electrical continuity from stub to top of the membrane with a continuous ribbon of dag.
11. Sputter-coat samples with gold or carbon using standard techniques and perform SEM, or store mounted samples with desiccant until it is convenient to coat them.

Safety Precautions

Glutaraldehyde, osmium tetroxide, and sodium cacodylate are all toxic and must be handled with standard laboratory precautions. Reagent solutions containing these chemicals should be prepared in a fume hood. The glutaraldehyde and osmium fixative solutions have volatile toxic components and should be dispensed under a fume hood. The procedure utilizing hexamethyldisilazane (HMDS) to replace critical point drying should be carried out in a fume hood.

Discussion

Permeable supports may either be fixed and dehydrated individually in separate wells of a Falcon® Multiwell Plate (as recommended) or processed batch-wise in a large petri dish or other vessel.

To achieve the most accurate preservation of surface features such as intercellular connections, ruffles, microvilli, and lamellipodia, efforts should be made to maintain constant pH and osmolality throughout the fixation procedure, including the secondary fixation in osmium tetroxide. Since glutaraldehyde apparently produces very low effective osmotic pressure (the membranes of many cell types are very permeable to GA), all buffer washes should have the same osmolality as the fixative vehicle solution, and this osmolality should be closely matched to that of the culture medium in which the cells are grown.[1] Buffers recommended in this protocol should produce good results for a variety of cell cultures grown in complete media having total osmolality around 300 mOsmol.

References

1. Brunk, U., Collins, P., Arros, E., Jrnl. Microscopy **123**:121 (1981).
2. Nation, J. L., Stain Tech., **58**:347 (1983).
3. Giammara, B., Proc. EMSA, **45**:878 (1987).
4. Pitt, A. and Gabriels, J., Am. Biotech. Lab., **4**:38 (1986).

Corning acquired the Falcon® brand.
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