Preparation of Transwell® Inserts for Histology

Guidelines for Use

CORNING

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Materials and Methods

- Phosphate buffered saline (PBS) pH 7.4 (Corning Cat. No. 21-040-CM)
- 4% Paraformaldehyde (PFA) in PBS pH 7.4 (alternative: 10% neutral buffered formalin pH 7.4)
- Ethanol (70%, 95%, and 100%)
- Scalpel blade
- Embedding cassettes
- Paraffin or other embedding media (heated to appropriate melting temperature)
- Xylene substitute (VWR Cat. No. 89370-090)*
- Positively charged or chrome-alum coated slides
- Staining solution such as eosin Y or hematoxylin
- 5% glacial acetic acid in distilled water
- 0.1% sodium bicarbonate in distilled water
- Resinous mounting medium (Mercedes Medical Cat. No. 7722)
- No. 1.5 cover glass (visit www.corning.com/lifesciences for preferred size cover glass)
- Forceps
- Lens paper
- Permeable Supports (e.g., Corning Cat. Nos. 3378, 3392, 3415, 3422)

Fixation

1. Do not touch either surface of the membrane. Remove culture medium from the well and rinse the Transwell inserts by adding PBS to the top of the membrane; then add PBS to the receiver plate until it reaches the membrane. Repeat the rinse procedure by first removing PBS from the receiver plate.
2. In a chemical fume hood, fix the cells by adding enough 4% PFA to cover the cultured cells. Fix for 15 to 30 minutes at room temperature.
3. Rinse off the 4% PFA with PBS twice and replace with 70% ethanol.

Preparation for Processing

1. Label an embedding cassette with pencil.
2. To remove the membrane from the insert, use forceps and pick up the Transwell insert and flip upside down. Cut the membrane off by using a sharp scalpel. Slowly cut around the inner circumference of the membrane. If the membrane starts to curl, adjust the angle or speed of the cutting to reduce the curling effect.
3. Place the membrane in lens paper and wrap it up by folding four times.
4. Place the wrapped membrane in an embedding cassette and return it to 70% ethanol.
5. Place embedding cassettes in a closed wide mouth container covering them with 70% ethanol.

Processing, Embedding, and Sectioning

1. Process the embedding cassettes in 10-minute steps of increasing grades of alcohol (i.e., 70%, 80%, 2 x 95%, 3 x 100%) and two changes of clearing solvent.
2. Infiltrate in two changes of melted paraffin for 30 minutes each.

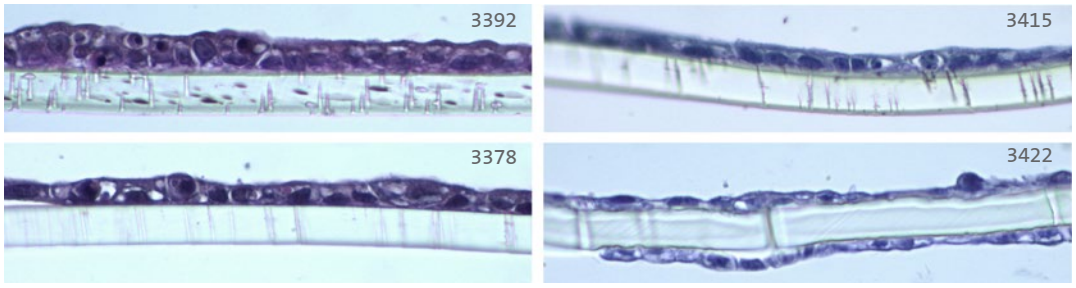
*Tested or recommended solvents: HistoChoice® (Amresco Cat. No. H1031L), EMS Xylene Substitute (Electron Microscopy Sciences Cat. No. 23410-01), and CitriSolv™ (Fisher Scientific Cat. No. 04-355-121). We do not recommend xylene-based clearing solvents.

3. At the embedding station, cut membranes in half with a microtome blade using one continuous straight movement. This reduces the degree of disturbance to the cells on the membrane. Embed on edge with cut surfaces down and slightly diagonal in the mold (so that the membrane is nearly perpendicular to the microtome blade).
4. Section at 4 to 5 microns and transfer sections to a distilled water bath warmed to 42°C to 44°C. Collect sections using positively charged or chrome-alum coated slides for maximum adhesion potential.
5. Bake slides in a 60°C oven for at least one hour.

Staining Procedure

Staining of sections can be done with any number of stains. A standard, routine hematoxylin and eosin (H&E) procedure is given below.

1. Deparaffinize sections by placing them in three changes of clearing solvent for 5 minutes each.
2. Rehydrate in two changes each of 100% and 95% ethanol for 2 minutes each.
3. Wash in gently running tap water for 2 to 3 minutes.
4. Place slides in the hematoxylin solution for 3 to 4 minutes.
5. Wash in gently running tap water for 2 to 3 minutes until water runs clear.
6. Place in 5% glacial acetic acid water for 1 minute.
7. Wash in gently running tap water for 2 minutes.
8. Place in 0.1% sodium bicarbonate for 1 minute.
9. Wash in gently running tap water for 2 minutes.
10. Place slides in 95% ethanol for 1 minute.
11. Place slides in eosin Y solution for 15 seconds.
12. Place slides in three changes of 95% ethanol for 0.5 to 1 minute each.
13. Place slides in three changes of 100% ethanol for 0.5 to 1 minute each.
14. Clear slides in three changes of clearing solvent for 3 minutes each.
15. Mount sections with 1 to 2 drops of mounting medium and carefully place cover glass over the section while trying to avoid air bubbles. Allow the slide to dry completely before visualizing (this can take up to 24 hours).



Representative images of MDCK cells on various membranes prepared with protocol (400X total magnification).

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