Preparation of Costar® Transwell® Inserts for Histology

Supplies

Reagents

10% Phosphate Buffered Formalin (pH 7.4) (Polysciences Cat. # 08379)
Ethanol, 95% and 100%
Hank's Balanced Salt Solution
Histoclear (RA lamb, Tyco Healthcare)
Paraffin or Paraplast
Harris Hematoxylin
Eosin Y
Li_2CO_3

Materials

Embedding molds (Polysciences Cat. # 02615)
Slides and cover slips
Mounting medium (Cytoseal 60) -Cat.# 18007
Oven set at 58°C

Protocol

1. Rinse the Transwell membrane with cells by adding HBSS (37°C) to the bottom of the well plate until it reaches the membrane, and then add HBSS to the top of the membrane. The rinse should be added slowly and then aspirated. Do not touch either surface of the membrane. Repeat the rinse procedure.

2. Add enough buffered formalin (room temperature) to the well and the membrane insert to cover the cultured cells. Fix for 1 hour at room temperature. This procedure should be conducted in a fume hood.

3. Rinse the membrane inserts with HBSS (37°C).

4. Dehydrate the insert by replacing the rinse with ethanol in a graded series of concentrations as follows:
   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 minutes

5. Remove the 100% ethanol and replace it with Histoclear (clearing agent). Allow the Histoclear to infiltrate the cells and membranes for 10 minutes. Repeat this step twice.

6. Infiltration of the cells and membrane is continued by removing the Histoclear from the membrane insert and adding liquid paraffin or Paraplast (58°C) to the bottom of the well plate and to the Transwell insert. Place it in an oven set at 58°C for one hour. Change the paraffin (Paraplast) and allow it to infiltrate in the oven for another hour.

7. Remove the well plate with the Transwell insert from the oven and allow it to solidify.

8. Remove the membrane insert by warming the outside of the well.

9. Remove the membrane (with attached paraffin) from the insert by cutting it out with a small, clean scalpel blade. Embedding the cells on the membrane while it is in the Transwell insert prevents damage to the cells from manipulation of the loose membrane.

10. The membrane plug can be embedded into a paraffin boat as if it were a piece of tissue. Keep in mind the orientation at which it is to be sectioned.

11. Sections are cut (5 to 10 microns thick) according to the standard procedures, mounted on glass slides, and allowed to dry.

Staining

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

1. Slides with sections are cleared of paraffin by placing it in Histoclear 3 times, 5 minutes per rinse.

2. Dip the slide in 100% ethanol 3 times.

3. Dip the slide in 95% ethanol twice.

4. Dip the slide in distilled water.

5. Place the slide in the hematoxylin solution for 9 minutes.
6. Gently rinse the slide with running tap water for 2 minutes.
7. Dip the slide in acid alcohol (1% HCl in 70% ethanol) 3 times.
8. Rinse the slide in distilled water.
9. Dip the slide 5 to 7 times in saturated Li_2CO_3 (approximately 10% w/v).
10. Rinse the slide in running tap water for 2 to 7 minutes.
11. Place the slide in eosin solution for 2 to 7 minutes.
12. Dip the slide in 95% ethanol 10 times.
13. Dehydrate the slide by placing it in 100% ethanol 3 times, 1 minute each time.
14. Clear the slide by placing it in Histoclear 4 times, 2 minutes each time.
15. Cover the sections with a drop of mounting medium (Cytoseal 60) and carefully add a glass cover slip (avoid bubbles). Allow the slide to dry.

For additional product or technical information, please visit our web site at www.corning.com/lifesciences or call at 1 800 492-1110. International customers can call at 978 635-2200.

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