Fixation and Staining Procedure for Costar® Transwell® Inserts

Supplies

Reagents
Phosphate Buffered Saline (for cell culture)
2.5% Glutaraldehyde, EM Grade 1.06 (Polysciences)
0.5% Triton X-100
Gill's Hematoxylin No.1 (Polysciences) Filter through a vacuum filter with a 0.2µm membrane.
Acid alcohol: 0.5% Hydrochloric Acid in 70% ethanol
0.04%Ammonium Hydroxide

Materials
Fume hood
Pipette aid and pipettes
Modified cluster dishes (holes in the cover and base)
Small basin or container

Fixation and Staining Procedure

Pipette all reagents down the side of the Transwell insert so that the cell layer will not be disrupted.

1. Rinse the Transwell membrane with cells by adding PBS (37°C) to the bottom of the well plate until it reaches the membrane, and then add PBS 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. Pipette enough PBS to cover surface of the membrane. PBS will remove any residual growth media. Remove the PBS from the Transwell insert.

Caution: From this point on, all work must be performed in the fume hood and gloves and safety glasses or goggles must be worn.

3. Pipette enough 2.5% EM grade glutaraldehyde to cover the surface of the membrane. Leaving the glutaraldehyde in the Transwell insert, incubate at room temperature for 15 minutes. Remove glutaraldehyde by carefully aspirating it or pouring it off.

4. Pipette enough 0.5% Triton X-100 to cover the surface of the membrane. Leaving the Triton X-100 in the Transwell insert, incubate for three minutes at room temperature. Remove Triton X-100 from the Transwell insert in the manner described in step 3.

5. Pipette enough Gill's hematoxylin No.1 to cover the surface of the membrane. Leaving the hematoxylin in the Transwell insert, incubate at room temperature for 15 minutes. Remove the hematoxylin from the Transwell insert in the manner described in step 3.

6. Rinse Transwell insert 3 to 4 times in a basin filled with distilled water to remove the excess stain.

7. Pipette enough acid alcohol to cover the surface of the membrane and leave it for 2 to 3 minutes to remove any residual stain (destain).

8. Rinse Transwell insert twice in a basin filled with fresh distilled water.

9. Pipette enough 0.04% NH_4OH to cover the surface of the membrane and leave it until a blue color is observed on the membrane (2-3 minutes).

10. Rinse Transwell insert twice in a basin filled with distilled water.

11. Air dry at an angle on a clean paper towel overnight.

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.

Corning Incorporated
Life Sciences

45 Nagog Park
Acton, MA 01720
t 800.492.1110
t 978.635.2200
f 978.635.2476

www.corning.com/
lifesciences

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f (55-11) 3167-0700

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f (52-81) 8313-8589