Immunofluorescence Staining of Human Endothelial Cells on a Gel Matrix Using the µ-Slide I Luer 3D

General information: This Application Note is a protocol for staining human umbilical vein endothelial cells (HUVEC) on a Collagen Type I gel using the ibidi µ-Slide I Luer 3D. As an example, the F-actin cytoskeleton, tubulin, and the nuclei are labelled for fluorescence microscopy.

Important Note: Compared to standard protocols, immunofluorescence staining with cells in a gel matrix needs more incubation time and more washing steps. This is to ensure sufficient diffusion of dyes and antibodies into the gel and their removal from the gel.

Related Documents:
Application Note 60 µ-Slide I Luer 3D

Keywords:
Immunostaining, antibody, fluorescence microscopy, F-actin, phalloidin, tubulin, DAPI, endothelial cells, HUVEC, monolayer, barrier, 3-D matrix, Collagen Type I, Rat Tail, extracellular matrix, ECM

Material:

- µ-Slide I Luer 3D ibiTreat (ibidi, 87176) prepared with gel matrix and cell monolayer, see Application Note 60 for details
- Paraformaldehyde, 4% (Sigma-Aldrich, HT5011)
- Triton® X-100, 0.1% in PBS (Alfa Aesar, A16046)
- Monoclonal anti-alpha-Tubulin antibody, mouse, 1:1000 in PBS (Sigma-Aldrich, T5168)
- Anti-mouse IgG-Atto594, 2 µg/ml (Sigma-Aldrich, 76085)
- Phalloidin-iFluor 488 Reagent, 1 µg/ml (abcam, ab176753)
- DAPI, 1 µg/ml (Sigma-Aldrich, D9542)
- Fluorescence microscope with appropriate filter sets

1. Fixation and Permeabilization

The handling protocol for liquid exchange inside the channel can be found in the instructions of the µ-Slide I Luer 3D. In brief, the liquid in the channel is exchanged stepwise by rinsing/washing the channel with a volume of 150 µl. First, the new solution is added to one empty Luer port. Next, the old solution is aspirated from the opposite side. Thus, the channel itself never falls dry.

- Aspirate the culture medium from the Luer ports. Leave the channel filled.
- Rinse 3 times with 150 µl paraformaldehyde and incubate for 10 minutes at room temperature.
- Wash 6 times with PBS.

- Rinse 3 times with 150 µl Triton® X-100. Incubate for 10 min.
- Wash 3 times with PBS.

2. Staining

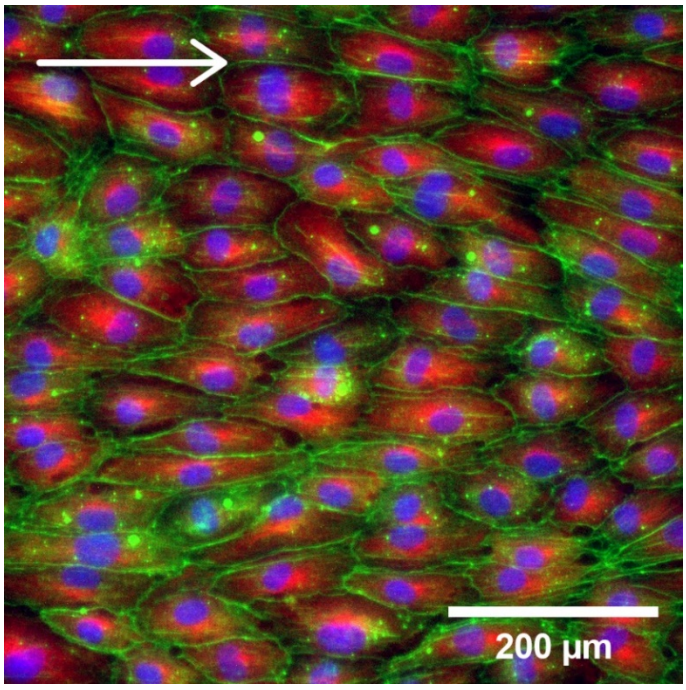
- Prepare the primary antibody mix (anti-Tubulin 1:1000 in PBS).
- Rinse 2 times with 150 µl primary antibody. Store overnight at 4°C in the dark.
- Wash 3 times with PBS. Incubate for 10 min.
- Wash 3 times with PBS.
- Prepare a secondary antibody mix containing Anti-mouse IgG-Atto594, Phalloidin-iFluor 488 Reagent, and DAPI in PBS.
- Rinse 2 times with 150 µl secondary antibody mix. Incubate for 3 hours at room temperature in the dark.
- Wash 3 times with PBS. Incubate for 10 min.
- Wash 3 times with PBS. Store overnight at 4°C in the dark.
- Wash 3 times with PBS.

3. Imaging

- Observe the cells under a fluorescence microscope with appropriate filter sets.
- Optionally, overlay images to create a merged image.

Important Note: Because of the height of the gel matrix, there are restrictions for objective lenses with a short working distance. Please refer to the Instructions of the µ-Slide I Luer 3D for details.

4. Results



Fluorescence microscopy of HUVEC after culturing them under flow at 10 dyn/cm² for 5 days on a Collagen Type I rat tail (2 mg/ml). Immunostaining of alpha-tubulin (red); the F-actin cytoskeleton was stained using phalloidin (green). Nuclei are stained with DAPI (blue). 10x objective.