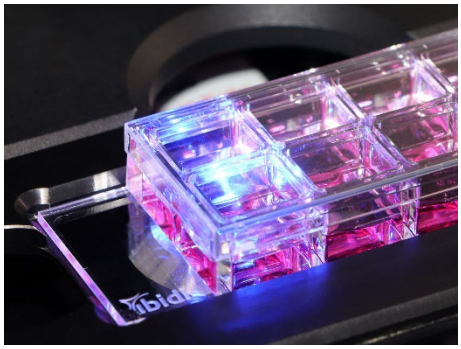
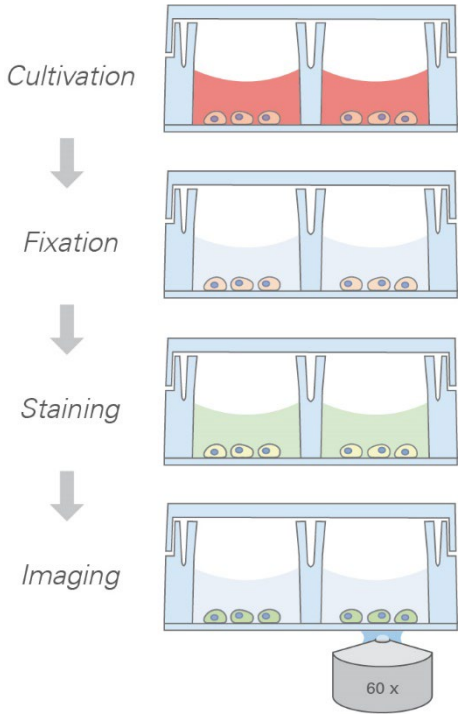
cells in focus ibidi®

Immunofluorescence Staining Using the µ-Slide 8 Well high



Immunofluorescence (IF) is a powerful approach for getting insight into cellular structures and processes using microscopy. This approach allows specific proteins to be assessed for their expression and location, making immunofluorescence indispensable for scientists to solve many cell-biological questions.

In this Application Note, we present a simple protocol for the cultivation and immunofluorescence staining of adherent human umbilical vein endothelial cells (HUVEC) in the µ-Slide 8 Well high.



Please note: before starting an immunocytochemistry experiment, several important parameters need to be checked, such as the expression level of the protein of interest, the optimal cell density, and the ideal cell culture vessel geometry and substrate/coating. In addition, the optimal antibody dilution should be evaluated as well. Further, positive and negative controls are necessary to validate the IF staining. Therefore, we strongly recommend a thorough literature research before the experiment.

Find more detailed information about planning and conducting immunofluorescence assays here.

1. Materials

1.1. Reagents and Buffers

Cell Culture

- Human umbilical vein endothelial cells (HUVEC, C-12203, Promocell)
- Collagen IV (354233, Corning)
- 0.05M HCl (X942.1, Carl Roth)
- Cell culture medium: Endothelial Cell Basal Medium (C-22210, Promocell) with Endothelial Cell Growth Medium Supplement Mix (C-39215, Promocell)
- PBS (14190144, Gibco)
- Accutase (A1110501, Gibco)

Immunofluorescence Staining

- PBS (14190144, Gibco)
- Formalin, 10%, ready to use (HT5011, Sigma Aldrich)
- Monoclonal anti-α-Tubulin (T5168, Sigma Aldrich)
- anti-mouse-IgG-Atto 594 (76085 Sigma Aldrich)
- Phalloidin 488 Conjugate (ab176753, Abcam)
- ibidi Mounting Medium With DAPI (50011, ibidi)
- Triton-X-100 (A16046, Thermo Fisher Scientific)
- Perforation Buffer (0.5% Triton-X-100 in PBS)
- Blocking Buffer (1% BSA + 0.2% Triton X 100 in PBS)
- Antibody Dilution Buffer (1% BSA + 0.05% Triton X 100 in PBS)
- Bovine Serum Albumin (BSA) (A1470-10G, Sigma Aldrich)
- ibidi Immersion Oil (50101, ibidi)

1.2. Equipment

- µ-Slide 8 Well ^{high}, ibiTreat (80806, ibidi)
- µ-Slide Rack (80003, ibidi)
- Standard cell culture equipment (pipettes, sterile working bench, cell culture incubator, culture flasks, cell culture medium, hemocytometer, etc.)
- Inverted fluorescence microscope with appropriate filter sets

2. Methods

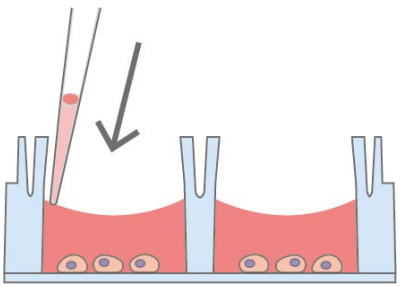
2.1. Cell Cultivation

Please read the Instructions before working with the µ-Slide 8 Well ^{high}, ibiTreat. Perform all steps under sterile conditions. Before starting the experiment, prepare the HUVECs in a standard cell culture flask (e.g., T75, coated with 6.7 µg/ml Collagen IV in 0.05 M HCl for 1 h) with the cells adherent at the bottom. The cells should be healthy and optimally subconfluent on the day of the experiment.

It is important to work swiftly during the whole procedure so the wells will not dry out.

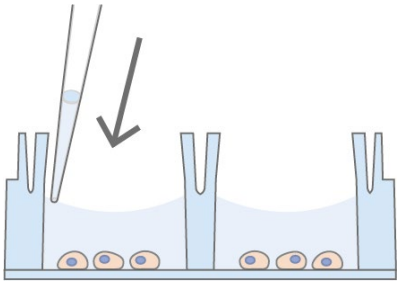
If not stated otherwise, all given volumes are per well, and all incubation steps are at room temperature.

- Treat the cultured HUVECs with Accutase for 1–2 min for detachment.
- Harvest the cell suspension, centrifuge, and dilute it in a low amount of culture medium for counting; the amount depends on the required cell concentration.
- Count the cells and adjust to a final concentration of 2 x 10^5 cells/ml in Endothelial Cell Basal Medium with Endothelial Cell Growth Medium Supplement Mix.
- Unpack an ibidi µ-Slide 8 Well ^{high} and put it on a µ-Slide Rack or an appropriate surface.
- Apply 250 µl of the HUVEC suspension into each well.
- Cover with the supplied lid.



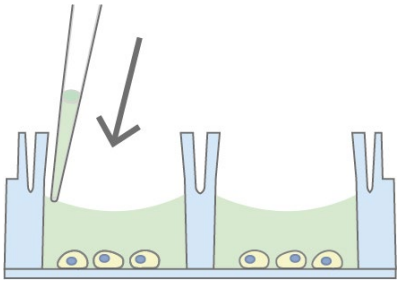
- Put the slide with the rack into the incubator (37°C, 5% CO_2) and let the cells attach for at least 3 h or overnight.
- For extended cell cultivation, we recommend a medium exchange every second day.

2.2. Fixation, Permeabilization, and Blocking

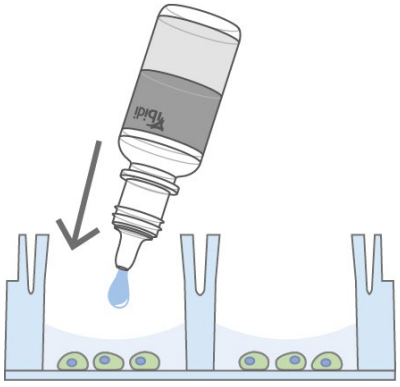


- Prepare enough Perforation Buffer and Blocking Buffer for your experiment.
- Aspirate the cell culture medium from the wells using a cell culture aspiration device.
- Wash the cells with PBS: slowly apply 200 µl PBS into each well and aspirate.
- Fix the cells with 200 µl formalin (10%) for 10 min.
- Remove the formalin and wash the cells three times with 200 µl PBS.
- Incubate the cells in 200 µl Perforation Buffer for 10 min.
- Remove the Perforation Buffer and wash the cells with 200 µL PBS.
- Block with 200 µl Blocking Buffer for 30 min.

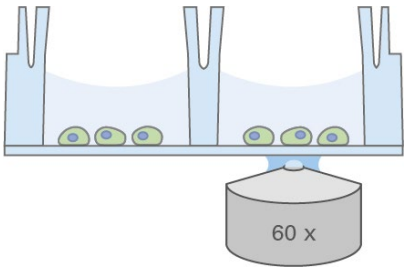
2.3. Staining and Mounting



- During the blocking step, prepare enough Antibody Dilution Buffer to dilute the primary and secondary antibodies.
- Dilute the primary antibody in Antibody Dilution Buffer (α-Tubulin: 1:200 dilution).
- Incubate the cells in 150 µl of the primary antibody solution for 2 h (please note that many antibodies require overnight incubation at 4°C).
- Wash twice with 200 µl Blocking Buffer.
- Dilute both the secondary antibody and the phalloidin in the same Antibody Dilution Buffer (anti-mouse-IgG-Atto 594: 1:200 dilution; Phalloidin 488 Conjugate 1:1000 dilution).
- Incubate the cells in 150 µl of the secondary antibody solution for 2 h in the dark. From this point on, the samples should be kept in the dark whenever possible.
- Wash twice with 200 µl Blocking Buffer.
- Empty all wells.
- Add one drop of ibidi Mounting Medium with DAPI per well.
- Store at 4°C in the dark until imaging. Optimally, proceed immediately with imaging since longer storage periods could reduce image quality.



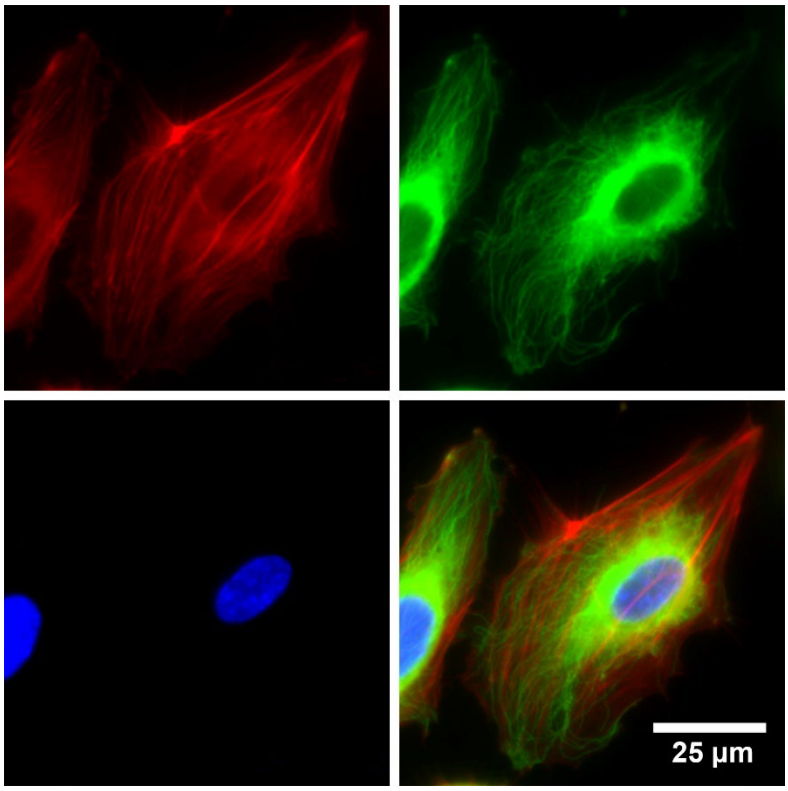
2.4. Imaging



- Observe the cells under a fluorescence microscope with appropriate filter sets and, if necessary, with ibidi Immersion Oil.
- Optionally, overlay images to create a merged image.

Which immersion oils are compatible with the ibidi labware products? Find out at: ibidi.com/oil

3. Results



Widefield fluorescence microscopy of immunostained HUVECs in a µ-Slide 8 Well high. The F-actin cytoskeleton was stained with phalloidin (red). α-Tubulin antibodies were used to stain the microtubules (green). Nuclei were visualized with DAPI (blue). The image was taken on a Nikon microscope using a 60x objective lens with oil immersion.

Your IF staining did not work out as expected? You can find troubleshooting tips here.