Immunofluorescence Staining Using the µ-Slide 18 Well

This Application Note presents a simple protocol for the cultivation, fixation, and staining of adherent cells using ibidi's µ-Slide 18 Well. In this example, we cultivated human endothelial cells, fixed them with paraformaldehyde, and stained the F-actin cytoskeleton and alpha-tubulin expressing microtubules. The nuclei were counterstained with DAPI.

Related Topics:

Application Note 09 Immunofluorescence Staining with µ-Slide VI 0.4

Application Note 16 Immunofluorescence Staining with µ-Slide 8 Well

Keywords:

Immunofluorescence, Fixation, Staining, Mounting, Microscopy, Cell Culture, HUVECs, F-actin, alpha-tubulin, DAPI, Chambered coverslip

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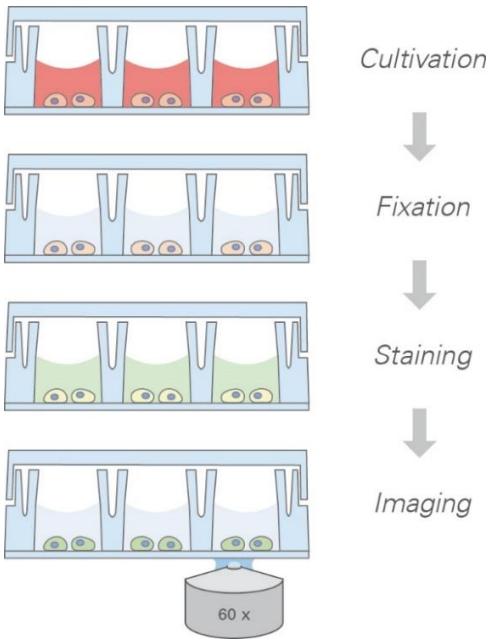
0. General Information

The protocol consists of four main steps:



In this application note, the following material is used:

Material	Manufacturer	Catalog number
µ-Slide 18 Well ibiTreat	ibidi	81816
HUVEC	various	various
4% Paraformaldehyde	Sigma-Aldrich	HT5011
0.1% Triton® X-100 (diluted in PBS)	Alfa Aesar	A16046
1% Bovine Serum Albumin (diluted in PBS)	Sigma-Aldrich	A1470
LifeAct-TagGFP2 Protein	ibidi	60112
Monoclonal anti-alpha-Tubulin antibody, mouse	Sigma-Aldrich	T5168
Anti-mouse IgG-Atto594	Sigma-Aldrich	76085
ibidi Mounting Medium with DAPI	ibidi	50011
Optional: ibidi Immersion Oil	ibidi	50101
Fluorescence microscope (inverted) with appropriate filter sets		

1. Cultivation

- Unpack the ibidi µ-Slide 18 Well ibiTreat (#81816) under sterile conditions and put it on a µ-Slide Rack (#80003).
- Prepare the cell suspension (1 x 10^5 cells/ml) and apply 100 µl into each well of the µ-Slide 18 Well.
- Cover the chambers with the supplied lid.
- Cultivate overnight in a humid cell culture incubator (37°C, 5% CO_2). For longer cell cultivation, we recommend a medium exchange after a few days.

2. Fixation, Permeabilization & Blocking

- Aspirate the cell culture medium from the wells using a cell culture aspiration device.
- Wash cells with Dulbecco's PBS by slowly applying 100 µl into each well.
- Fix cells with ~100 µl of 4% paraformaldehyde for 20 min.
- Wash cells twice with PBS by slowly applying 100 µl into each well.
- Remove the liquid and apply ~100 µl of 0.1% Triton® X-100 in PBS. Incubate for 10 min.
- Wash cells with PBS by slowly applying 100 µl into each well.
- Remove the liquid and apply 100 µl 1% BSA blocking solution in PBS. Incubate for 20 minutes.
- Wash cells with PBS by slowly applying 100 µl into each well.

3. Staining

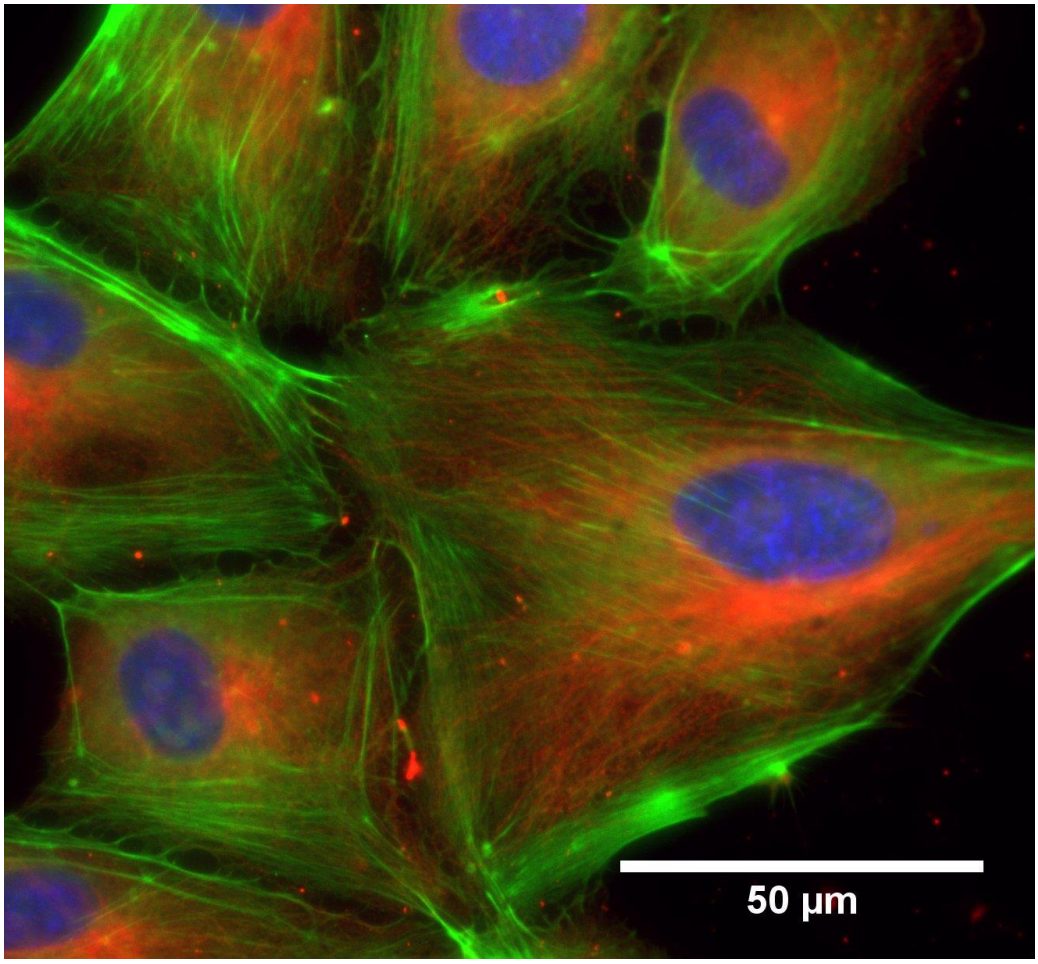
- Prepare your staining and antibody solutions.
- Remove all liquid from the wells using a cell culture aspiration device.
- Apply 100 µl of primary antibody (anti-Tubulin 1:1000) diluted in PBS into each well and incubate overnight at 4 °C.

- Wash the cells once with PBS for 10 minutes.
- Apply 100 µl of secondary antibody mix (Anti-mouse IgG-Atto594 1:500 and LifeAct-TagGFP2 Protein 30 µg/ml) diluted in PBS into each well and incubate for 3 hours at room temperature in the dark.
- Wash the cells twice with PBS for 10 minutes.
- Aspirate the PBS and add ~100 µl ibidi Mounting Medium with DAPI into each well. Use the dropper bottle to add the Mounting Medium dropwise.

4. Imaging

- Observe the cells under a fluorescence microscope with appropriate filter sets and optionally with ibidi Immersion Oil (#50101).
- Optionally, overlay images to create a merged image.

5. Results



HUVEC (Human umbilical vein endothelial cell) 24 hours after seeding in µ-Slide 18 Well, ibiTreat, objective lens 60x, oil immersion.

Green: F-Actin (LifeAct-TagGFP2 protein)

Red: Tubulin (monoclonal anti-alpha-Tubulin antibody, mouse + Anti-mouse IgG-Atto594)

Blue: Nuclei (DAPI staining using ibidi Mounting Medium with DAPI)