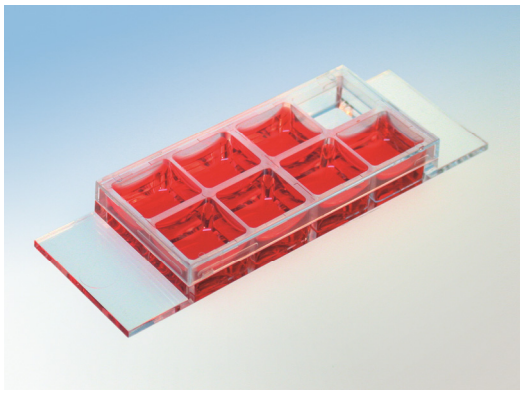
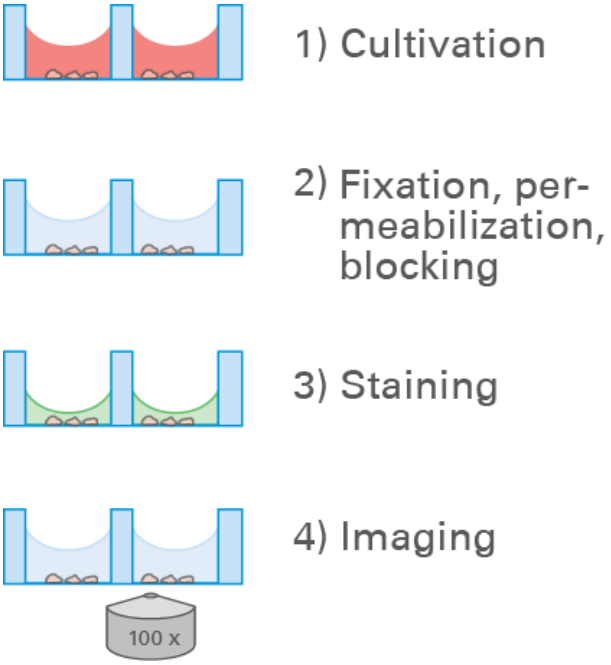
Immunofluorescence Staining with µ-Slide 8 well



In this Application Note we present a simple protocol for cultivation and staining of adherent cells in ibidi's µ-Slide 8 well. In this example we cultivated rat fibroblasts, fixed them with paraformaldehyde, stained the mitochondria with MitoTracker and counterstained the nucleus with DAPI. All commonly used fixation techniques may also be used. Additionally, with primary and secondary antibody stainings, it is possible to probe for other intracellular structures by immuno-cytochemistry.

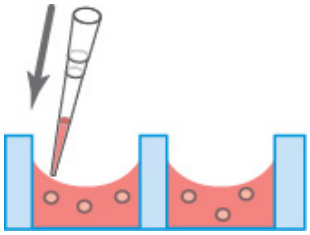
The protocol consists of four main steps:



In our example we used the following materials:

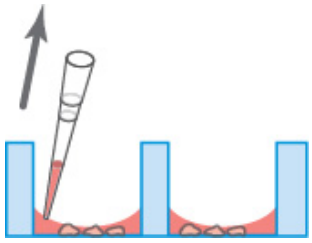
- Rat fibroblasts (cell line)
- µ-Slide 8 well, ibiTreat (ibidi, 80826)
- Paraformaldehyde (2% in PBS)
- Triton® X-100 (Fluka, 0.1%)
- Blocking solution (1% BSA in PBS)
- MitoTracker Green (Life Technologies, 50 nM)
- DAPI (Sigma, 0.1 µg/ml)
- Fluorescence microscope (inverted) with appropriate filter sets

1) Cultivation

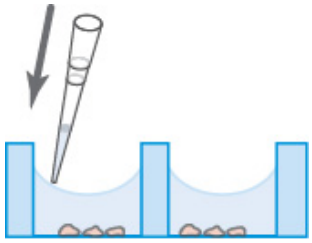


- Unpack an ibidi µ-Slide 8 well, ibiTreat (80826) under sterile conditions and put it on a µ-Slide Rack (80003) or an appropriate surface. Apply 300 µl of a $5x10^4$ cells/ml cell suspension into each well.
- Cover with the supplied lid.
- Put the slide with the rack into the incubator (37°C; 5% CO_2) and let cells attach. Incubate for at least 3 h or over night.
- For longer cell cultivation we recommend a medium exchange after some days.

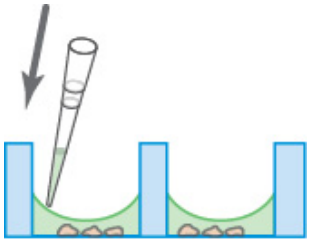
2) Fixation, Permeabilization, and Blocking



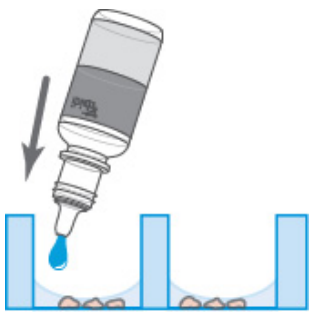
- Aspirate the cell culture medium from the wells using a cell culture aspiration device.
- Wash cells with Dulbecco's PBS by slowly applying 300 µl into each well.
- Fix cells with ~150 µl of 2 % para-formadehyde in PBS.
- After 20 min remove the liquid and apply ~150 µl of 0.1% Triton® X-100.
- After 10 min remove the liquid and wash cells with 300 µl BSA blocking solution.



3) Staining and Mounting

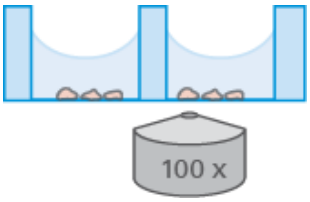


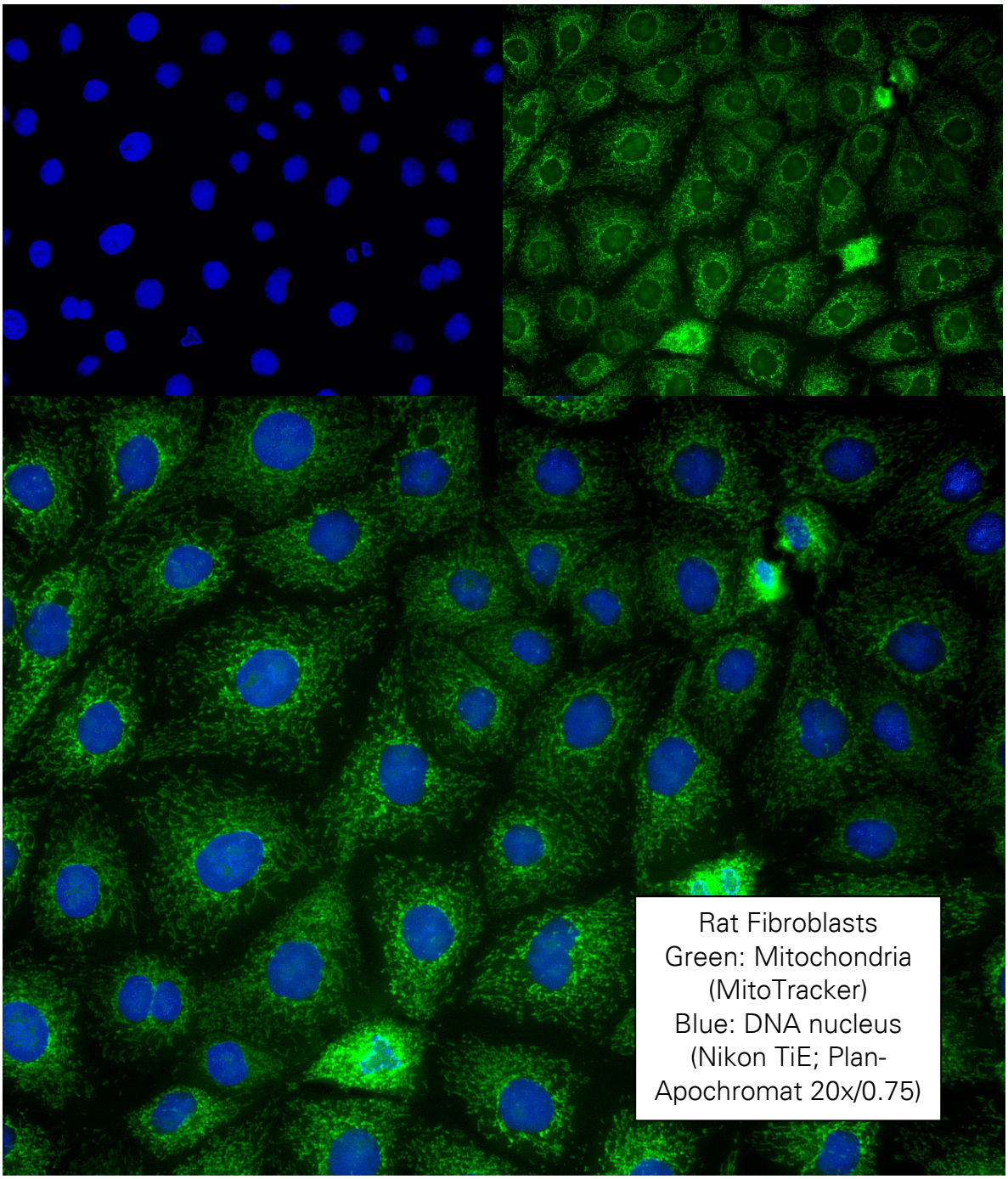
- Prepare your staining and antibody solutions.
- Apply 150 µl of the DAPI solution and incubate at room temperature for 30 min.
- Wash cells twice with 300 µl BSA blocking solution as described above.
- Apply 150 µl of the MitoTracker solution and incubate for 45 min.
- Wash cells twice with 300 µl BSA blocking solution as described above.
- Empty all wells.
- Add ~150 µl of ibidi Mounting Medium dropwise as shown.



4) Imaging

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





Rat Fibroblasts
Green: Mitochondria (MitoTracker)
Blue: DNA nucleus
(Nikon TiE; Plan-Apochromat 20x/0.75)