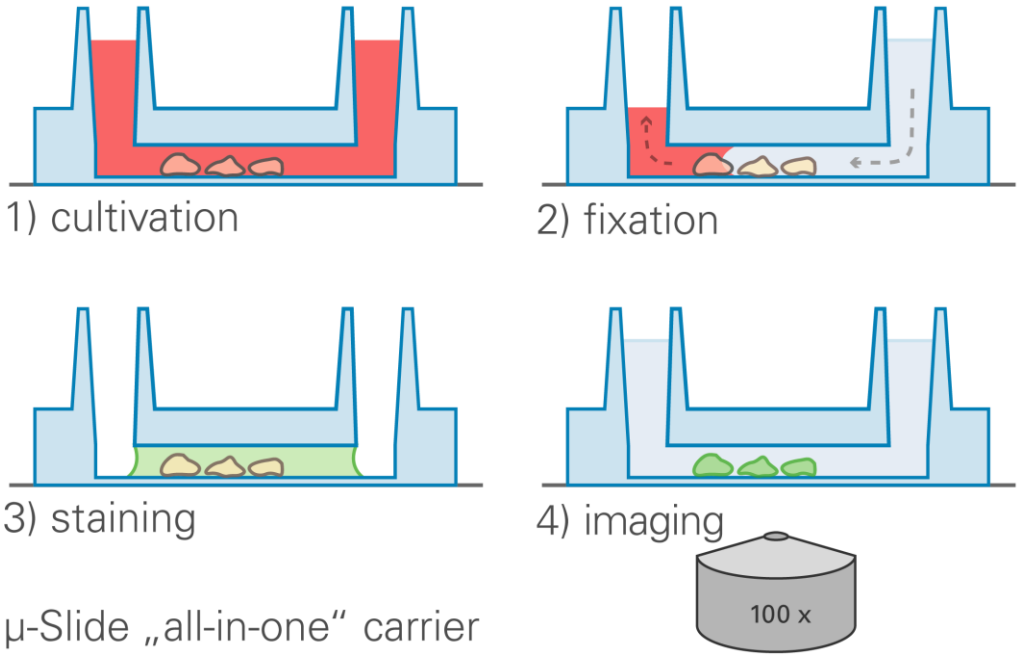
Cell cultivation and immunofluorescence staining with µ-Slide VI 0.4

In this protocol we describe a single example of cultivating HT-1080 cancer cells inside the µ-Slide VI $^{0.4}$. Subsequently, we stained the F-actin cytoskeleton with Alexa Fluor® 488 phalloidin and counterstained the nucleus with DAPI.

The protocol consists of four main steps:

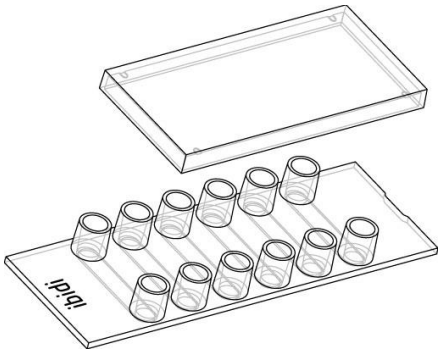


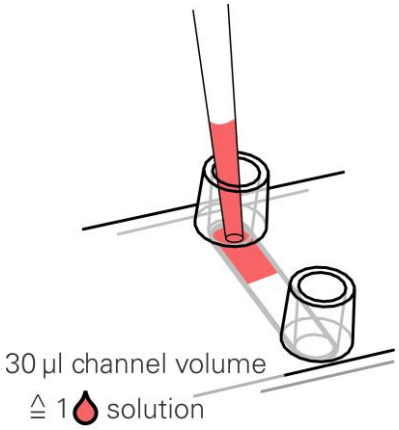
Material needed:

- HT1080 cell suspension (180 µl; 3x10^5 cells/ml)
- DMEM with 10% FCS
- µ-Slide VI $^{0.4}$ with ibiTreat surface (ibidi #80606)
- Dulbecco´s PBS
- 3.7 % paraformaldehyde (PFA) in PBS
- 0.1% Triton® X-100
- 1% BSA in PBS
- Alexa Fluor® 488 phalloidin (0.165 µM)
- DAPI (0.1 µg/ml)
- ibidi Mounting Medium (ibidi #50001)
- optional: ibidi Immersion Oil (ibidi #50101)

1) Cultivation

- Unpack a µ-Slide VI $^{0.4}$, ibiTreat (80606) under sterile conditions and put it on a µ-Slide rack (80003).
- Apply 30 µl of a 3x10^5 cells/ml HT-1080 cell suspension into each channel. Pipet directly into the channel as illustrated below or shown on our website.





- Cover reservoirs with the supplied lid.
- Put the slides with the rack into the incubator (37 °C; 5 % CO_2) and let cells attach (60 min). Afterwards fill both reservoirs with 60 µl of cell-free medium.
- Incubate over night.

2) Fixation and Permeabilization of cells

During fixation and permeabilization take care, that the channel never falls dry! Exchange the liquid in the channel by flushing the remaining solution out with the next one.

Fixation:

- Aspirate medium from all reservoirs using a cell culture aspiration device. Wash cells with Dulbecco´s PBS by slowly applying 200 µl into one empty reservoir of each channel and aspirating from the opposite reservoir for each channel. Don't aspirate the entire channel volume.
- Fix cells with ~100 µl of 3.7 % paraformaldehyde in PBS. After 20 min flush the liquid inside the channel by filling one well with 200 µl PBS and removing the content of the reservoir from the other well; ensuring the channel is never dry.

Permeabilization and blocking

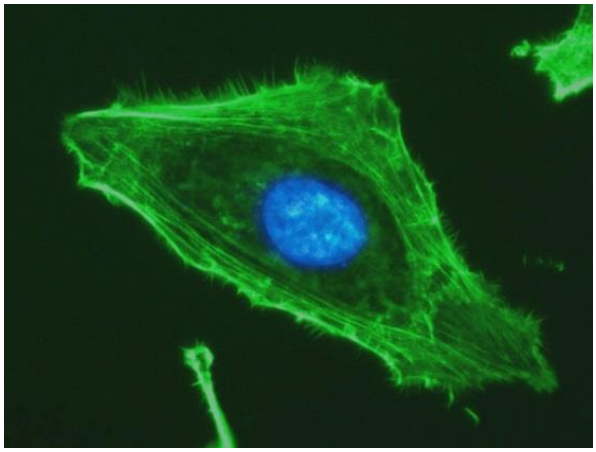
- Wash cells again with 200 µl PBS as described above.
- Apply ~100 µl of 0.1% Triton® X-100 in PBS for 3-5 min.
- Wash cells with PBS.
- Apply ~100 µl blocking solution (1% BSA in PBS) for 20 min.
- Wash cells with PBS.

3) Staining

- Remove all liquid from the channel using your aspiration device. Don´t let the channel dry out.
- Right after aspirating, apply 30 µl of Alexa Fluor® 488 phalloidin (1 Unit in 200 µl PBS with 1% BSA). It can be helpful to inject the solution with a 1 ml syringe. Incubate at room temperature for 20 min.
- Wash cells with PBS.
- Apply 30 µl DAPI (0.1 µg/ml) for 3-5 min.
- Wash cells with PBS and apply ibidi Mounting Medium until the channel is filled (ca. 50 µl). ibidi Mounting Medium is glycerol-based and contains DABCO for anti-fading. The slide can be stored for approx. 4 weeks.

4) Imaging

- Observe cells under a fluorescence microscope with appropriate filter sets and optionally with immersion oil.
  Please note! The ibidi standard bottom is compatible with certain types of immersion oil only (e.g. ibidi Immersion Oil, #50101). A list of all suitable oils can be found in the µ-Slide instructions.



HT-1080 cell;
green: F-actin cytoskeleton;
blue: nucleus;
(Zeiss Axiovert 135;
Plan-Neofluar 40x/0.75)