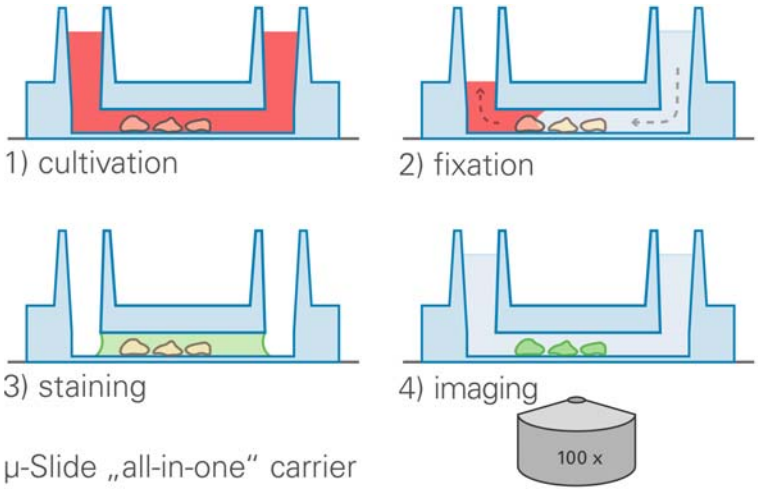
Cell cultivation and immunofluorescence staining with µ-Slide VI 0.4

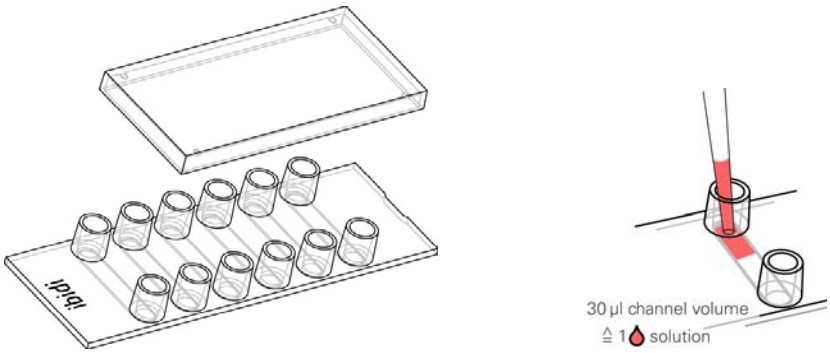
In this protocol we describe a single example of cultivating HT-1080 cancer cells inside the µ-Slide VI $^{0.4}$. Subsequently, we stained the F-actin cytoskeleton with Alexa Fluor® 488 phalloidin and counterstained the nucleus with DAPI.

The protocol consists of four main steps:



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 of cells

- 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 % para-form-aldehyde in PBS. After 10 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 (Fluka) in PBS for 3-5 min.
- Wash cells with PBS.
- Apply ~100 µl of 1% BSA in PBS solution 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.**

- Right after that, apply **25 µl** of Alexa Fluor® 488 phalloidin (1 Unit + 500 µl PBS + 1% BSA, Invitrogen Corp.) Incubate at room temperature for 20 min.

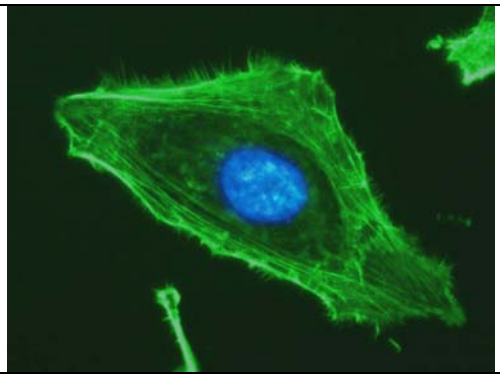
- Wash cells with PBS.

- Apply **25 µl** DAPI (0.1 µg/ml, Sigma-Aldrich) 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.



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

* As published: Lee J.H., Koh H., Kim M., Kim Y., Lee S.Y., Karess R.E., Lee S.-H., Shong M., Kim J.-M., Kim J. & Chung J.; Energy-dependent regulation of cell structure by AMP-activated protein kinase. Nature Aug **2007**; doi:10.1038/nature05828