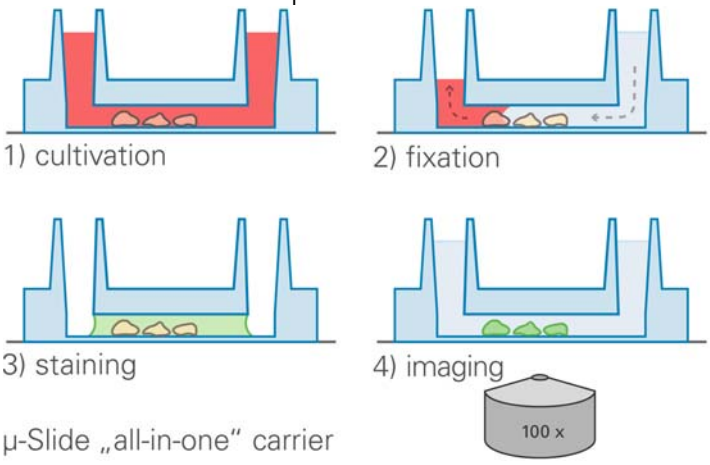
Application note for cell cultivation and immunofluorescent staining in µ-Slide y-shaped

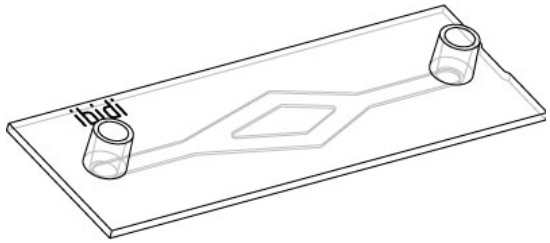
A protocol for cultivation and staining of HUVEC in ibidi µ-Slide y-shaped. In this example we stained the von-Willebrand-Factor (vWF) on human umbilical vein endothelial cells (HUVEC) with Cy5 ®, the cluster of differentiation molecule 31 (CD31) with Alexa Fluor 488 ® with an indirect staining process and we counterstained the nucleus with DAPI.

The protocol consists of four main steps:



1) Cultivation

- Unpack an ibidi µ-Slide y-shaped, ibiTreat (80126) under sterile conditions and put it on a µ-Slide rack (80003). Apply 200 µl of a $1x10^6$ cells/ml HUVEC suspension into the channel and remove the liquid from the reservoirs. Pipet directly into the channel illustrated below.



- Cover reservoirs with the supplied lids.
- Put the slide with the rack into the incubator (37°C; 5% CO_2) and let cells attach (approx. 60 min). Afterwards fill both reservoirs with 60 µl of cell-free medium.
- Incubate for at least 3hrs or over night.
- Connect the slide to the ibidi pump system (consisting of: ibidi perfusion set, ibidi fluidic unit, ibidi air pressure pump) under sterile conditions and cultivate cells under flow in an incubator (37°C; 5% CO_2). We recommend a shear stress of 7.5 dyne/cm².
- With 11ml of media a media exchange of 6 ml has to be done within 3 days.

2) Fixation, permeabilization and blocking of cells

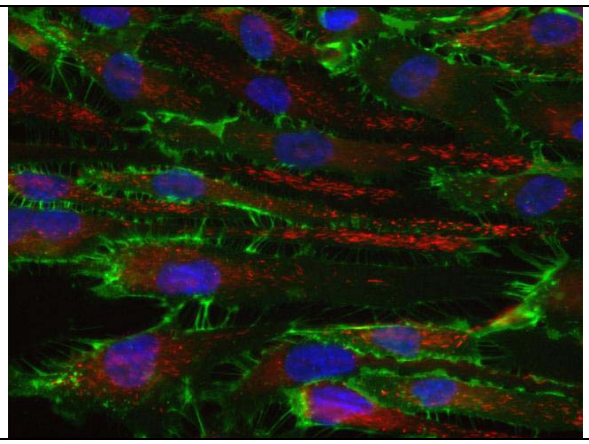
- Aspirate medium from all reservoirs using a cell culture aspiration device. Wash cells with Dulbecco´s PBS by slowly applying 1000 µl into one reservoir while aspirating from the opposite reservoir. **Caution:** The channel volume has to be filled with liquid the entire time to avoid bubbles and loss of cells.
- Fix cells with ~150 µl of 2% paraformadehyde in PBS. After 20 min remove the liquid inside the channel by filling one well with ~150µl of 0.1% Triton® X-100 (Fluka) in PBS while aspirating the liquid of the second reservoir! Make sure the channel is never dry!
- Wash cells with 1000µl 1%BSA in PBS as described above.

3) Staining and mounting

- Prepare your antibody solution beforehand (we used: DAPI in a concentration of 0,1µg/ml; anti-vWF (rabbit) (Sigma) and anti-CD31 (mouse) (Sigma) 1:1000; anti-rabbit (goat) Cy5® (1mg/ml) (Invitrogen corp.) 1:150 and anti-mouse (chicken) Alexa Fluor® 488 (2mg/ml) (Invitrogen corp.) 1:200)
- Apply 200µl of DAPI (Sigma) in PBS and 1%BSA into the channel and incubate at room temperature (rt) for 30min.
- Wash cells twice with 1000µl 1%BSA in PBS as described above
- Apply 200µl of anti-vWF (rabbit) in PBS and 1%BSA into the channel and incubate at rt for 45min.
- Wash cells twice with 1000µl 1%BSA in PBS as described above
- Apply 200µl of anti-CD31 (mouse) in PBS and 1%BSA into the channel and incubate at rt for 45min.
- Wash cells twice with 1000µl 1%BSA in PBS as described above
- Apply 200µl of anti-mouse (chicken) Alexa Fluor® 488 and incubate at rt for 30min.
- Wash cells twice with 1000µl 1%BSA in PBS as described above
- Apply 200µl of anti-CD31 (mouse) and incubate at rt for 30min.
- Wash cells twice.
- Wash cells with with 1000µl 1%BSA in PBS as described above and apply 200 µl of glycerol (80% in PBS with 2% added DABCO) for mounting and 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.



HUVEC;
green: CD 31(PECAM-1);
red: Factor VIII (vWF);
blue: DNA 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