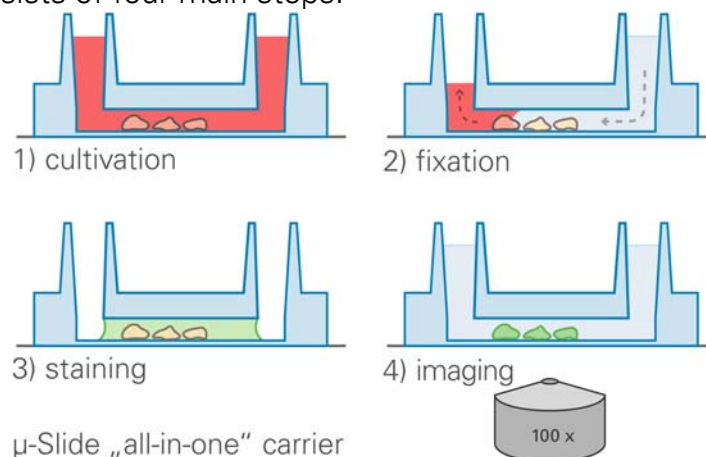


## Application note for cell cultivation and immunofluorescent staining in $\mu$ -Slide y-shaped

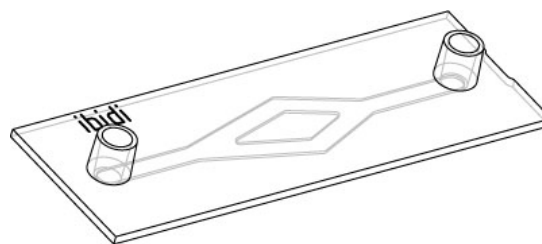
A protocol for cultivation and staining of HUVEC in ibidi  $\mu$ -Slide y-shaped. In this example we stained the von-Willebrand-Factor (vWF) on human umbilical vein endothelial cells (HUVEC) with Cy5<sup>®</sup>, the cluster of differentiation molecule 31 (CD31) with Alexa Fluor 488<sup>®</sup> with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

The protocol consists of four main steps:



### 1) Cultivation

- Unpack an ibidi  $\mu$ -Slide y-shaped, ibiTreat (80126) under sterile conditions and put it on a  $\mu$ -Slide rack (80003). Apply 200  $\mu$ l of a  $1 \times 10^6$  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<sub>2</sub>) and let cells attach (approx. 60 min). Afterwards fill both reservoirs with 60  $\mu$ l 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<sub>2</sub>). We recommend a shear stress of 7.5 dyne/cm<sup>2</sup>.
- With 11ml of media a media exchange of 6 ml has to be done within 3 days.

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### 2) Fixation, permeabilization and blocking of cells

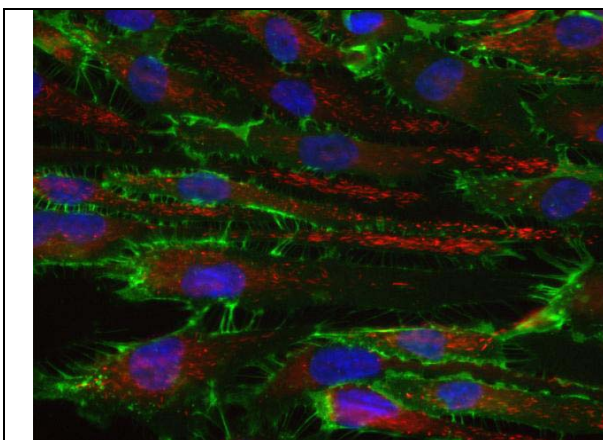
- Aspirate medium from all reservoirs using a cell culture aspiration device. Wash cells with Dulbecco's PBS by slowly applying 1000  $\mu$ l into one reservoir while aspirating from the opposite reservoir. **Caution:** The channel volume has to be filled with liquid the entire time to avoid bubbles and loss of cells.
- Fix cells with ~150  $\mu$ l of 2% paraformaldehyde in PBS. After 20 min remove the liquid inside the channel by filling one well with ~150 $\mu$ l 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 1000 $\mu$ l 1%BSA in PBS as described above.

### 3) Staining and mounting

- Prepare your antibody solution beforehand (we used: DAPI in a concentration of 0,1 $\mu$ g/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 200 $\mu$ l of DAPI (Sigma) in PBS and 1%BSA into the channel and incubate at room temperature (rt) for 30min.
- Wash cells twice with 1000 $\mu$ l 1%BSA in PBS as described above
- Apply 200 $\mu$ l of anti-vWF (rabbit) in PBS and 1%BSA into the channel and incubate at rt for 45min.
- Wash cells twice with 1000 $\mu$ l 1%BSA in PBS as described above
- Apply 200 $\mu$ l of anti-CD31 (mouse) in PBS and 1%BSA into the channel and incubate at rt for 45min.
- Wash cells twice with 1000 $\mu$ l 1%BSA in PBS as described above
- Apply 200 $\mu$ l of anti-mouse (chicken) Alexa Fluor® 488 and incubate at rt for 30min.
- Wash cells twice with 1000 $\mu$ l 1%BSA in PBS as described above
- Apply 200 $\mu$ l of anti-CD31 (mouse) and incubate at rt for 30min.
- Wash cells twice.
- Wash cells with with 1000 $\mu$ l 1%BSA in PBS as described above and apply 200  $\mu$ l 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