Application note for cell cultivation and immunofluorescent staining with µ-Slide I

In this protocol we describe a single example of cultivating fibroblasts of rat (Rat1) inside the µ-Slide I. 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:

Seeding cells

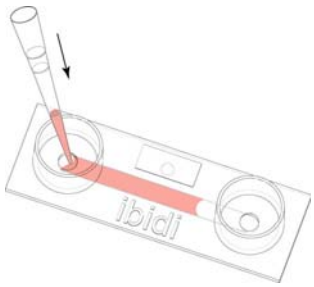
Fixation of cells

Permeabilization and blocking

Staining

Seeding cells

1) Unpack a µ-Slide I, ibiTreat (80106) under sterile conditions and put it on a µ-Slide rack (80003). Apply 100 µl of a $3x10^5$ cells/ml Rat1 cell suspension into the channel. Pipet directly into the channel as illustrated below or shown on our website.



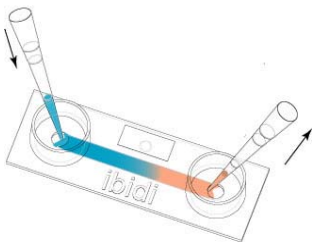
2) Cover reservoirs loosely with the supplied caps. Do not close them completely.

3) Put the rack into the incubator (37°C; 5% CO_2) and let cells attach (60 min). Afterwards fill both reservoirs with 0.5 ml of cell-free medium.

4) Incubate over night.

Fixation of cells

5) Aspirate the entire medium from the reservoirs by using a cell culture aspirating device or a pipet. Wash cells with Dulbecco´s PBS by slowly applying 1 ml into one empty reservoir and aspirating from the opposite reservoir. Don't aspirate the entire channel volume.



6) Fix cells with the same procedure by using ~200 µl of 3.7% para-formaldehyde in PBS. Remove the content of the reservoirs from the opposite side of the channel and wait for 10 min.

Permeabilization and blocking

7) Wash cells again with 1 ml PBS as described in step 5.

8) Apply ~200 µl of 0.1% Triton® X-100 (Fluka) in PBS for 3-5 min.

9) Wash cells with PBS.

10) Apply ~200 µl of 1% BSA in PBS solution for 20 min.

11) Wash cells with PBS.

Staining and mounting

12) Remove all liquid from the channel. Don´t let the channel dry after aspirating the liquid from the channel.

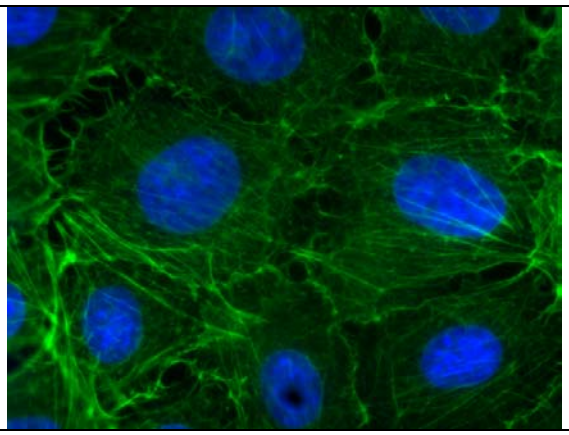
13) Apply 100 µl of Alexa Fluor® 488 phalloidin (1 Unit + 500 µl PBS + 1% BSA, Invitrogen Corp.) Incubate at room temperature for 20 min.

14) Wash cells with PBS and empty the channel.

15) Apply 100 µl DAPI (0.1 µg/ml, Sigma-Aldrich) for 3-5 min.

16) Wash cells with PBS and remove all the liquid. Apply 100 µl of glycerol (80% in PBS with 2% added DABCO) for mounting and anti-fading*. Cover reservoirs with the supplied caps. The slide can be stored for approx. 4 weeks.

17) Observe cells under a fluorescence microscope with appropriate filter sets and optionally with immersion oil.



Rat1 cells;
green: F-actin cytoskeleton;
blue: nucleus
(Zeiss Axiovert 135; Plan-Neofluar 100x/1.3)

* 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