

## **Application note for cell cultivation and immunofluorescent staining with $\mu$ -Slide I**

In this protocol we describe a single example of cultivating fibroblasts of rat (Rat1) inside the  $\mu$ -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**

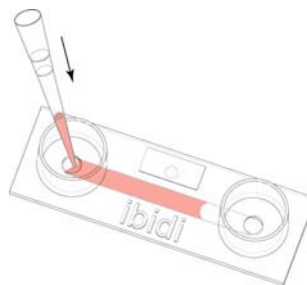
### **Fixation of cells**

### **Permeabilization and blocking**

### **Staining**

#### **Seeding cells**

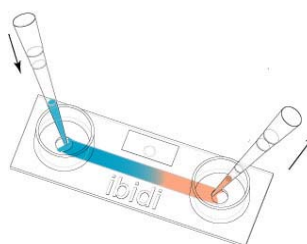
- 1) Unpack a  $\mu$ -Slide I, ibiTreat (80106) under sterile conditions and put it on a  $\mu$ -Slide rack (80003). Apply 100  $\mu$ l of a  $3 \times 10^5$  cells/ml Rat1 cell suspension into the channel. Pipet directly into the channel as illustrated below or shown on our website.



- 2) Cover reservoirs loosely with the supplied caps. Do not close them completely.
- 3) Put the rack into the incubator (37°C; 5% CO<sub>2</sub>) 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.



## Application Note 02

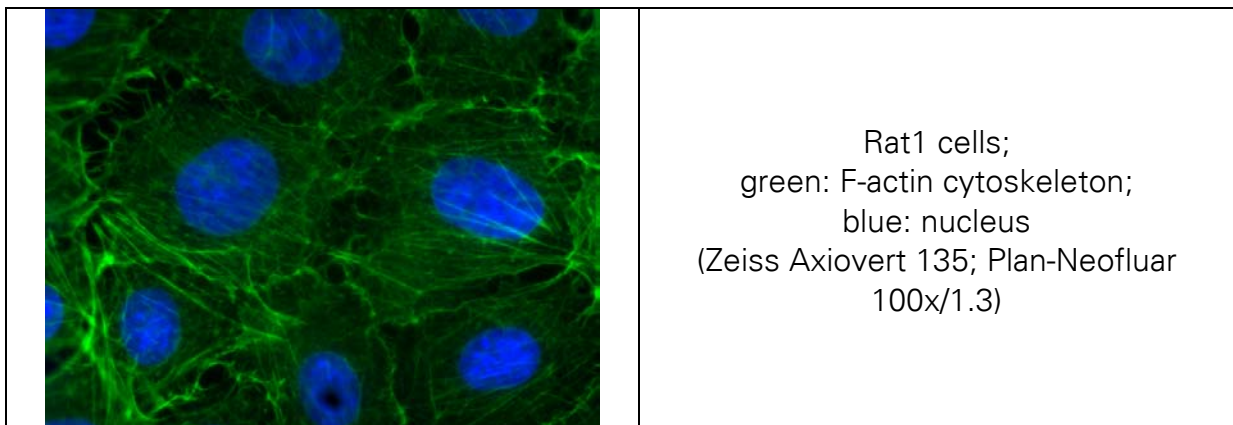
- 6) Fix cells with the same procedure by using ~200  $\mu$ l of 3.7% para-formaldehyde in PBS. Remove the content of the reservoirs from the opposite side of the channel and wait for 10 min.

### Permeabilization and blocking

- 7) Wash cells again with 1 ml PBS as described in step 5.
- 8) Apply ~200  $\mu$ l of 0.1% Triton® X-100 (Fluka) in PBS for 3-5 min.
- 9) Wash cells with PBS.
- 10) Apply ~200  $\mu$ l 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  $\mu$ l of Alexa Fluor® 488 phalloidin (1 Unit + 500  $\mu$ l PBS + 1% BSA, Invitrogen Corp.) Incubate at room temperature for 20 min.
- 14) Wash cells with PBS and empty the channel.
- 15) Apply 100  $\mu$ l DAPI (0.1  $\mu$ g/ml, Sigma-Aldrich) for 3-5 min.
- 16) Wash cells with PBS and remove all the liquid. Apply 100  $\mu$ l 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.



\* 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