# Instructions





The ibidi product family comprises a variety of different shapes of  $\mu$ -Slides,  $\mu$ -Dishes and plates which all have been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength. The  $\mu$ -Slide I combines the features of a cell culture dish with those of a glass cover slip. One flow through observation channel is integrated in the  $\mu$ -Slide. Its large observation area and high-end optical quality permits convenient monitoring of a vast variety of cellular assays.

# Material

The  $\mu$ -Slides consist of a plastic with highest optical quality. The material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the slide. The  $\mu$ -Slides are not autoclavable since they are temperature stable up to 60°C/140°F only. Please note that gas exchange between the channel and incubator's atmosphere occurs partially through the plastic bottom which should not be covered. Thus, it is recommended to place the  $\mu$ -Slide on an ibidi  $\mu$ -Slide rack which can be purchased from your local distributor.

#### **Immersion oil**

When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the objective.

| company  | product                            | ordering number   |  |  |
|----------|------------------------------------|-------------------|--|--|
| Cargille | type DF, Formula<br>Code: 1261     | (Cargille) 16242  |  |  |
| Zeiss    | 518 F                              | (Zeiss) 444960    |  |  |
| Olympus  | 50CC                               | (Olympus) 35506   |  |  |
| Nikon    | 50 CCM DF                          | (Nikon) MXA 20351 |  |  |
| Leica    | immersion oil, low<br>fluorescence | (Leica) 11513859  |  |  |

# µ-Slide surfaces

Depending on your cells and special application you will need  $\mu$ -Slides with different surfaces. If you do not need any special adhesion molecules for your application the best choice will be ibiTreat, a tissue culture treated surface. We provide precoated  $\mu$ -Slides with adhesion substrates like Collagen IV, Fibronectin, Poly-L-Lysin, and Poly-D-Lysin. Such adhesion substrates have been shown to stimulate adhesion and growth of various cell lines in  $\mu$ -Slides. Only high quality substrates are used <sup>1</sup>.

The uncoated  $\mu$ -Slide is manufactured from hydrophobic plastic. For cultivation of most cell lines it is indispensable to treat the uncoated  $\mu$ -Slide with biopolymers which mediate cell adhesion and growth.

# Coating your µ-Slide I

The uncoated  $\mu$ -Slide must be coated to promote cell adhesion. If you like to establish a certain coating for your demands we recommend to test your coating procedure on uncoated and ibiTreat  $\mu$ -Slides, since we have observed that some biomolecules adhere differently to hydrophobic or hydrophilic plastic surfaces.

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply 100 µl of the solution as it is shown in the movie manuals on www.ibidi.com. Leave at room temperature at least 30 minutes.
- Aspirate the solution and wash with 1 ml ultra-pure water. Let dry at room temperature.

Further information about coatings are provided in Application Note 08 Cell culture coating.

# Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a  $3-7 \times 10^5$  cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 100  $\mu$ l cell suspension into the channel of the  $\mu$ -Slide. Quick dispensing helps to avoid trapped air bubbles. Fill 600  $\mu$ l cell free medium into each reservoir. Please avoid tilting of the  $\mu$ -Slide in order to prevent the cells from being flushed out of the channel.
- Cover reservoirs loosely with the supplied caps. Incubate at 37°C and 5 % CO<sub>2</sub> as usual.

<sup>1</sup>Collagen IV, BD Cat.-Nr. 35 6233, Fibronectin, BD Cat.-Nr. 354008, Poly-L-Lysin, Sigma Cat.-Nr. P4832, Poly-D-Lysin, BD Cat.-Nr. 354210



# Tip:

The day before seeding the cells we recommend to place the cell medium and the  $\mu$ -Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Trapped bubbles can be removed from the channel by inclining the  $\mu$ -Slide and knocking at one edge.

In order to achieve maximal homogeneity of cell distribution, fill the channel with 100  $\mu$ l cell suspension and cover reservoirs with caps. Await cell attachment. Afterwards fill 600  $\mu$ l cell free medium into each reservoir.

Further information are provided in Application Note 03 Growing cells in µ-channels.

# **Exchanging medium**

Aspirate both reservoirs and fill slowly 1.2 ml of fresh medium into one of the reservoirs, which will replace the channel volume by gravity flow.

#### Preparation for cell microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the  $\mu$ -Slide on an inverted microscope. You can use any fixative of your choice. The  $\mu$ -Slide material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180  $\mu$ m, high resolution microscopy is possible.

For flow applications we recommend using µ-Slide I Luer

# µ-Slide I family

The µ-Slide I family is available with different surfaces. See table below for choosing your µ-Slide I.

| Ordering number | Treatment or Coating                                | characteristics                     |
|-----------------|---|-------------------------------------|
| 80106           | ibiTreat, tissue culture treated, sterile           | hydrophilic, tissue culture treated |
| 80101           | Collagen IV, sterile                                | protein coating                     |
| 80102           | Fibronectin, sterile*                               | protein coating                     |
| 80110           | Poly-L-Lysine, sterile                              | biopolymer coating                  |
| 80115           | Poly-D-Lysine, sterile*                             | biopolymer coating                  |
| 80111           | uncoated, sterile                                   | hydrophobic                         |
| 80141           | elastic surface ESS, 28 kPa, sterile**              | hydrophobic                         |
| 80142           | elastic surface ESS, 28 kPa, Collagen IV, sterile** | protein coating                     |

\* available on request only

\*\* the ESS surface is supported by a glass cover slip No. 1.0. Please also refer to the ESS instructions.



#### Selected cell tests on different surfaces

Many eukaryotic and bacterial cells have been tested by ibidi on the different surfaces of the µ-Slides. A variety of other cell lines like COS, CHO, HepG3, and NIH 3T3 were successfully grown by our customers.

|                          | ibiTreat  | Collagen IV | Fibronectin | Poly-L-Lysin   | Poly-D-Lysin | uncoated       |
|--------------------------|-----------|-------------|-------------|----------------|--------------|----------------|
| HUVEC                    | excellent | good        | excellent   | no cell growth | not done     | no cell growth |
| Rat1                     | excellent | excellent   | excellent   | excellent      | excellent    | poor           |
| HT1080                   | excellent | excellent   | excellent   | excellent      | not done     | poor           |
| HeLa                     | excellent | excellent   | excellent   | excellent      | not done     | poor           |
| Neuro2A                  | excellent | excellent   | excellent   | excellent      | excellent    | poor           |
| PC12                     | good      | excellent   | excellent   | excellent      | excellent    | no cell growth |
| Dictyostelium discoideum | not done  | excellent   | not done    | not done       | not done     | excellent      |
| Escherichia coli         | excellent | not done    | not done    | excellent      | not done     | excellent      |

HUVEC = Human Umbilical Vein Endothelial Cells Rat1 = Rat Fibroblast HT1080 = Human Fibrosarcoma HeLa = Human Cervix Adenocarcinoma Neuro2A = Mouse Neuroblastoma

PC12 = Rat Pheochromocytom

*Dictyostelium discoideum* = strain wild type AX-2

*Escherichia coli* = strain MDG131

For research use only!

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