



The ibidi product family comprises a variety of different shapes of μ -Slides and μ -Dishes 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 μ -Slide VI ^{0.4} can be connected to a pump and enables you to observe cells under flow

conditions. Alternatively, the convenient six channel format is also ideal for static cell cultivation and the application of standard protocols for e.g. treatment, staining, and microscopy of living or fixed cells.

Material

ibidi μ -Slides and μ -Dishes 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 upper part. The μ -Slides and μ -Dishes are not autoclavable since they are temperature stable up to 60°C/140°F only. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the plastic bottom which should not be covered.

Geometry of the μ -Slide VI ^{0.4}

The μ -Slide VI ^{0.4} provides standard slide format according to ISO 8037/1. The lateral adapter to adapter distance of 9 mm (like 96 well plates) allows using multichannel pipettes.

| Dimensions | | | | |
|--------------------------|---------------------------------|--|--|--|
| Number of channels | 6 | | | |
| Channel volume | 30 µl | | | |
| Channel length | 17 mm | | | |
| Channel width | 3.8 mm | | | |
| Channel height | 0.4 mm | | | |
| Adapters | female Luer | | | |
| Volume per reservoir | 60 µl | | | |
| Growth area | 0.6 cm ² per channel | | | |
| Coating area using 30 µl | 1.2 cm ² per channel | | | |
| Bottom matches coverslip | No. 1.5 | | | |

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 plastic material and the objective.

| Company | Product | Ordering number |
|----------|------------------------------------|-------------------|
| Cargille | type DF, Formula Code: 1261 | (Cargille) 16242 |
| Zeiss | 518 F | (Zeiss) 444960 |
| Olympus | 50CC | (Olympus) 35506 |
| Nikon | 50 CCM DF | (Nikon) MXA 20351 |
| Leica | immersion oil, low fluorescence | (Leica) 11513859 |

µ–Slide surfaces

Depending on your cells and special application you will need μ -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 μ -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 μ -Slides. Only high quality substrates are used ¹.

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

Coating your µ–Slide VI 0.4

The uncoated μ -Slide must be coated to promote cell adhesion. If you like to establish a certain coating for your demands we recommend testing your coating procedure on uncoated and ibiTreat μ -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.

¹Collagen 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



- Apply 30 µl per channel and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with ultra-pure water. You can add the water into one channel end and simultaneously aspirate it on the other side. 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 30 µl cell suspension into the channel of the µ–Slide. Quick dispensing helps to avoid trapped air bubbles.
- Cover reservoirs with the supplied lid. Incubate at 37° C and 5% CO₂ as usual.
- Await cell attachment in order not to flush out the cells. Afterwards fill each reservoir with 60 μ l cell free medium.

Tip:

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

Trapped air bubbles can be removed from the channel by inclining the μ -Slide and knocking at one edge.

Exchanging medium

Aspirate both reservoirs and fill slowly 120 μ l of fresh medium into one of the reservoirs, which will replace the channel volume by gravity flow.

Flow application

Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11 "Shear stress and shear rates" on <u>www.ibidi.com</u>.

Suitable flow kits (μ -Slide VI ^{0.4} + tubing and adapters) are also available.



Please contact us for recommended perfusion setups. ibidi provides a variety of pump systems.

Preparation for cell microscopy

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



Instructions

μ –Slide VI ^{0.4} family

The μ -Slide VI ^{0.4} family is available with different surfaces. See table below for choosing your μ -Slide VI ^{0.4}.

| Ordering number | Treatment or Coating | Characteristics |
|-----------------|---|-------------------------------------|
| 80606 | ibiTreat, tissue culture treated, sterile | hydrophilic, tissue culture treated |
| 80602 | Collagen IV, sterile | protein coating |
| 80603 | Fibronectin, sterile* | protein coating |
| 80604 | Poly-L-Lysine, sterile | biopolymer coating |
| 80605 | Poly-D-Lysine, sterile* | biopolymer coating |
| 80601 | uncoated, sterile | hydrophobic |

* available on request only

$\mu\text{--Slide VI}^{0.4}$ flow kits

The μ -Slide VI ^{0.4} is available as flow kit with tubes and adapters.

| alle alle | Ordering number | Treatment or Coating | Characteristics | | |
|-----------|-----------------|---|-------------------------------------|--|--|
| -992299 | 80646 | ibiTreat, tissue culture treated, sterile | hydrophilic, tissue culture treated | | |
| 12 100000 | 80642 | Collagen IV, sterile | protein coating | | |
| | 80641 | uncoated, sterile | hydrophobic | | |

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, HepG2, 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!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany. © ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.