

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 2x9 well harbours two arrays of 3x3 square fields where cells can be cultivated and investigated with microscopical methods. It is intended for checking out experimental parameters like antibody dilution, seeding

density or most effective drug concentrations. A special application is the co-cultivation of different cell types which share one medium supernatant but grow separately from each other.

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 80°C/175°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.

Optical properties ibidi standard bottom

Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 μm)
Material	microscopy plastic

Geometry

The μ-Slide 2x9 well provides standard slide format according to ISO 8037/1.

Geometry of μ-Slide 2x9 well

Number of major wells	2
Major wells (w × l × h) in mm	21.5 × 23.6 × 6.8
Volume per major well	600 μl
Number of minor wells	2 × 9
Minor wells (w × l × h) in mm	6.1 × 6.8 × 1.3
Volume per minor well	70 μl
Growth area per minor well	0.4 cm ²
Total height with lid	8 mm
Bottom matches coverslip	No. 1.5

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 2x9 well

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.
- Apply 70 μl per minor well (=1.26 ml per Slide) and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with ultra-pure water. Let dry at room temperature.

Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 5–10 × 10⁴ cells/ml suspension should result in a confluent layer within 2–3 days.

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

Instructions

μ-Slide 2x9 well

- Use the center minor well for recipient cells and the 8 outer wells for feeder cells.
- Apply 40-60 μl cell suspension into each minor well of the μ-Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover reservoirs with the supplied lid. Incubate at 37 °C and 5% CO₂ as usual.
- After cell attachment fill 400-600 μl into each large reservoir, allowing the cells to share factors.

Undemanding cells can be left in their seeding medium for up to three days and grow to confluency there. However, best results might be achieved when the medium is changed every 1-2 days. Carefully aspirate the old medium and replace by 1.2 ml fresh medium.

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 www.ibidi.com. Due to the thin bottom of only 180 μm, high resolution microscopy is possible.

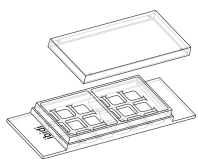
Immersion oil

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 2x9 well family

The μ-Slide 2x9 well family is available with different surfaces. See table below for choosing your μ-Slide 2x9 well.



Ordering number	Treatment or Coating	characteristics
81806	ibiTreat, sterile	hydrophilic, tissue culture treated
81802	Collagen IV, sterile	protein coating
81803	Fibronectin, sterile*	protein coating
81804	Poly-L-Lysine, sterile	biopolymer coating
81805	Poly-D-Lysine, sterile*	biopolymer coating
81801	uncoated, sterile	hydrophobic

* available on request only

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
<i>Dictyostelium discoideum</i>	not done	excellent	not done	not done	not done	excellent
<i>Escherichia coli</i>	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.

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