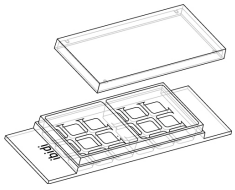


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength. The µ–Slide 2 × 9 well harbours two arrays of 3×3 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, µ–Dishes, and µ–Plates are made of a plastic that has the highest optical quality. The bottom material exhibits extremely low birefringence and autofluorescence, similar to that of glass. Also, it is not possible to detach the bottom from the upper part. The µ–Slides, µ–Dishes, and µ–Plates are not autoclavable, since they are only temperature–stable up to 80°C/175°F. 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 2 × 9 well provides standard slide format according to ISO 8037/1.

Geometry of µ–Slide 2 × 9 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 the type of cells and the special application you are using, you will need µ–Slides with different surfaces. If you do not require 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 the 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 the cultivation of most cell lines, it is indispensable totreat the uncoated µ–Slide with biopolymers, which mediatecell adhesion and growth.

Coating your µ–Slide 2 × 9 well

The uncoated µ–Slide must be coated to promote cell adhesion. If you want to establish a certain coating to match your needs, we recommend testing your coating procedure on both uncoated and ibiTreat µ–Slides, since we have observed that some biomolecules adhere differently to hydrophobic and 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 × 10^4 cells/ml

[1] 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

suspension should result in a confluent layer within 2–3 days.

- 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 % CO_2 as usual.
- After cell attachement 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.

Please also see our Application Note 10, "Co-Cultivation Using ibidi µ–Slides".

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.

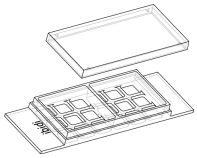
Immersion Oil

When using oil immersion objectives, use only the immersion oils specified in the table. The use of a non–recommended oil could lead to the damage of the plastic material and the objective.

Company	Product	Ordering Number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

µ–Slide 2 × 9 well family

The µ–Slide 2 × 9 well family is available with different surfaces. See table below for choosing your µ–Slide 2 × 9 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 References

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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 46 17 0. All products are developed and produced in Germany.