



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 18 well - flatflat is a convenient multi–well plate for quick and easy tests to probe experimental parameters like antibody dilution, seeding density or most effective drug concentrations. Unlike in 96 well plates, the whole area of the well can be observed in phase–contrast microscopy mode since there is no disturbing refraction of the liquid surface meniscus.

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	

Please note! The ibidi standard bottom is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 2.

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 indis-

pensable to treat the uncoated μ -Slide with biopolymers, which mediate cell adhesion and growth.

Geometry of the μ -Slide 18 well - flat

The μ -Slide 18 well - flatprovides standard slide format according to ISO 8037/1. The well to well distance of 9 mm (like 96 well plates) allows using multichannel pipettes.

Geometry of the μ -Slide 18 well - flat		
Number of wells	18	
Volume per reservoir	30 µl	
Well diameter	5 mm	
Growth area per well	0.2 cm^2	
Coating area per well	0.25cm^2	
Height with/without lid	5.0/1.6 mm	
Bottom matches coverslip	No. 1.5	

Coating your µ-Slide 18 well - flat

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 30 µl per well and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with the recommended protein dilution buffer.
- Optionally let dry at room temperature. Attention, some coating proteins might degenerate when dry-ing!

¹Collagen IV: Corning #356233, Fibronectin: Corning #354008, Poly–L–Lysin: Sigma #P4832, Poly–D–Lysin: Corning #354210

Instructions



Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a $2-4 \times 10^5$ cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 30 µl cell suspension into each well of the µ– Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover the slide with the supplied lid. Incubate at 37° C and 5% CO₂ as usual.

Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. However, best results might be achieved when the medium is changed every 1–2 days. Carefully aspirate the old medium and replace it by $30 \,\mu$ /well fresh medium.

The volume of a single well is very small. Depending on your cell type the medium might be consumed after some hours. If you want to incubate your cells for longer than a couple of hours we recommend to aspirate and refill cell medium every day.

Tip:

If the wells are properly filled with 30 μ l, the liquid surface is planar and in good alignment with the μ -Slides surface. This is how you will be able to observe the whole well area with unimpaired phase contrast.

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 preferably 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 μ m, 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 nonrecommended oil could lead to the damage of the plastic material and the objective.

Company	Product	Ordering Number
ibidi	Immersion Oil	(ibidi) 50101
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859



Instructions

µ-Slide 18 well - flatfamily

The μ -Slide 18 well - flatfamily is available with different surfaces. See table below for choosing your μ -Slide 18 well - flat.

Ordering Number	Treatment or Coating	Characteristics
81826	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
81822	Collagen IV, sterile	protein coating
81823	Fibronectin, sterile*	protein coating
81824	Poly–L–Lysine, sterile	biopolymer coating
81825	Poly–D–Lysine, sterile*	biopolymer coating
81821	uncoated, sterile	hydrophobic
81831	PEN-membrane, 1 μm, sterile**	for Laser Microdissection

* available on request only

** The PEN foil does not fit to standard cover slip thickness.



Selected References

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P. J. Tesar. Derivation of germ-line-competent embryonic stem cell lines from preblastocyst mouse embryos. *Proc Natl Acad Sci US A*, 2005.

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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 4617 0. All products are developed and produced in Germany. © ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.