

# Instructions µ-Slide 8 Well



The ibidi product family is comprised of a variety of  $\mu$ –Slides and  $\mu$ –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  $\mu$ –Slide 8 Well is an array of 8 square fields where cells can be cultivated and, subsequently, investigated with microscopical methods. It is intended for the optimization of experimental parameters like antibody dilution, seeding density, or the most effective drug concentration.

#### **Material**

ibidi  $\mu$ –Slides,  $\mu$ –Dishes, and  $\mu$ –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  $\mu$ –Slides,  $\mu$ –Dishes, and  $\mu$ –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 polymer coverslip, which should not be covered.

#### **Optical Properties ibidi Standard Bottom**

Refractive index n <sub>D</sub> (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 μm)
Material	microscopy plastic/ polymer coverslip

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.

#### Geometry

The  $\mu$ -Slide 8 well provides standard slide format according to ISO 8037/1.

Geometry of µ–Slide 8 Well		
Number of wells	8	
Dimensions of wells ( $w \times l \times h$ ) in mm	$9.4\times10.7\times6.8$	
Growth area per well	$1.0 \text{ cm}^2$	
Coating area per well	$2.2\mathrm{cm}^2$	
Recommended filling volume per well	300 µl	
Total height with lid	8 mm	
Bottom matches coverslip	No. 1.5	

#### **Shipping and Storage**

The  $\mu$ -Slides,  $\mu$ -Dishes and  $\mu$ -Plates are sterilized and welded in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is listed in the following table.

Conditions		
Shipping conditions	Ambient	
Storage conditions	RT (15-25°C)	
Shelf Life of Different Surfaces		
ibiTreat, Glass Bottom, ESS	36 months	
Collagen, Poly-Lysine	18 months	
Fibronectin	4 months	

#### μ-Slide Surfaces

Depending on the type of cells and the special application you are using, you will need  $\mu$ –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  $\mu$ –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  $\mu$ –Slides. Only high–quality substrates are used  $^1$ .

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

<sup>&</sup>lt;sup>1</sup>Collagen IV: Corning #356233, Fibronectin: Corning #354008, Poly–L–Lysin: Sigma #P4832, Poly–D–Lysin: Corning #354210



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#### Coating your µ-Slide 8 Well

The uncoated  $\mu$ –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  $\mu$ –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 300 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 drying!

Detailed information about coatings is 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  $5-11 \times 10^4$  cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 300 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  $37^{\circ}\text{C}$  and 5% CO<sub>2</sub> 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 300 µl/well fresh medium.

#### Tip:

As you may know from 96 well plates, the bent meniscus at the air–liquid interphase in small open wells destroys the phase contrast effect of your microscope image. To avoid this problem, we recommend using our channel Slides such as the  $\mu$ –Slides I Luer and  $\mu$ –Slide VI  $^{0.4}$  or a Ph+ Slide.

### **Preparation for Cell Microscopy**

To analyze your cells, no special preparations are necessary. Cells can be observed live, or fixed directly in the  $\mu\text{--}$  Slide on an inverted microscope. You can use any fixative of your choice. The  $\mu\text{--}$  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  $\mu\text{m}$ , 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 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



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## **Ordering Information**

The  $\mu$ –Slide 8 Well family comprises Slides with different surfaces and bottom characteristics. See table below for choosing your  $\mu$ –Slide 8 Well.

μ–Slide 8 Well



Cat. No.	Description
80826	μ–Slide 8 Well ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized
80822	μ–Slide 8 Well Collagen IV: #1.5 polymer coverslip, sterilized
80823	μ–Slide 8 Well Fibronectin: #1.5 polymer coverslip, sterilized*
80824	μ-Slide 8 Well Poly-L-Lysine: #1.5 polymer coverslip, sterilized
80825	<b>μ–Slide 8 Well Poly-D-Lysine</b> : #1.5 polymer coverslip, sterilized*
80821	μ–Slide 8 Well Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized

<sup>\*</sup> available on request only

#### μ-Slide 8 Well Glass Bottom



Cat. No.	Description
80827	μ–Slide 8 Well Glass Bottom: 1.5H (170 μm ±5 μm) D 263 M Schott glass, sterilized

### μ–Slide 8 Well Grid–500



Cat. No.	Description
80826-G500	$\mu$ –Slide 8 Well ibiTreat Grid–500: #1.5 polymer coverslip, tissue culture treated, grid repeat distance 500 μm, sterilized
80821-G500	$\mu$ –Slide 8 Well Uncoated Grid–500: #1.5 polymer coverslip, hydrophobic, grid repeat distance 500 μm, sterilized



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#### Selected References

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- J. Lazar, A. Bondar, S. Timr, and S. J. Firestein. Two-Photon Polarization Microscopy Reveals Protein Structure and Function. *Nature Methods*, 2011. doi: 10.1038/nmeth.1643.

#### 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.