# Instructions





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 glass bottom versions of the  $\mu$ -Slides and  $\mu$ -Dishes are especially designed for TIRF and single molecule applications. The  $\mu$ -Slide 4 well is an array of 4 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

The glass bottom version of the  $\mu$ -Slides are made of a standard  $\mu$ -Slide but with a glass coverslip bottom. It is not possible to detach the bottom. The  $\mu$ -Slides are not autoclavable since they are temperature stable only up to 80°C / 175°F.

Optical Properties ibidi glass bottom		
Refractive index $n_D$	1.523	
Abbe number	55	
Thickness	No. 1.5H (selected quality 170 μm, ± 5 μm)	
Material	Schott borosilicate glass, D 263M	

#### Geometry

The  $\mu$ -Slide 4 well provides a standard slide format according to ISO 8037/1.

Geometry of µ-Slide 4 well glas	s bottom
Number of wells	4
Dimensions of wells (w $\times$ l $\times$ h) in mm	21.2 × 11.0 × 9.3
Growth area per well	$2.2 \text{ cm}^2$
Coating area per well	$4.1 \text{ cm}^2$
Recommended filling volume per well	700 µl
Total height with lid	10.8 mm
Bottom matches coverslip	No. 1.5

#### Surface and coating

The  $\mu$ -Slide 4 well glass bottom is manufactured with an uncoated glass coverslip. Washing steps (e.g. with PBS) before cell seeding can remove glass dust which is advantageous for direct cell growth on the surface.

Protein coatings increase direct cell growth of adherent cells. Specific coatings on glass are possible following this protocol:

- Prepare your coating solution according to the manufacturer's specifications or reference. Prepare your  $\mu$ -Slide. Adjust the concentration to a coating area of  $4.1 \text{ cm}^2$  and 700  $\mu$ l.
- Apply 700 µl into the growth area. Make sure that the entire bottom is covered with liquid easily tilting or shaking the µ–Slide. Put on the lid 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!

#### 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 700 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  $37^{\circ}$ 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  $700 \,\mu$ /well fresh medium.



## Tip:

As you may know from the 96 well plates, a bent meniscus at the air-liquid interphase in small open wells will destroy the phase contrast effect of your microscope image. Use the Ph+ version to overcome this disturbing effect.

## Solvents for Fixation and Staining

Cells can be observed live or fixed directly in the  $\mu$ -Slide preferably on an inverted microscope. The slide material is compatible to acids, alkalis, PFA, and silicone oil. Alcohols may be used for short term incubation (e.g. cell fixation). Acetone is not compatible. Further specifications can be found at www.ibidi.com.

For optimal results in fluorescence microscopy and storage of stained probes ibidi provides a mounting medium (50001) optimized for  $\mu$ -Dishes and  $\mu$ -Slides.

#### **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.

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



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## µ–Slide 4 well Family

The  $\mu$ -Slide 4 well family is available as open well and as a Ph+ version. See table below for choosing your  $\mu$ -Slide 4 well.  $\mu$ -Slide 4 well

	Ordering Number	Treatment or Coating	Characteristics
ADA	80426	ibiTreat, sterile	hydrophilic, tissue culture treated
	80422	Collagen IV, sterile	protein coating
	80423	Fibronectin, sterile*	protein coating
	80424	Poly-L-Lysine, sterile	biopolymer coating
	80425	Poly-D-Lysine, sterile*	biopolymer coating
	80421	uncoated, sterile	hydrophobic
	80427	glass bottom	glass coverslip No. 1.5H (170 $\mu$ m ±5 $\mu$ m)

## $\mu$ –Slide 4 well <sup>Ph+</sup>

Ordering Number	Treatment or Coating	Characteristics
80446	ibiTreat, sterile	hydrophilic, tissue culture treated
80442	Collagen IV, sterile	protein coating
80443	Fibronectin, sterile*	protein coating
80444	Poly-L-Lysine, sterile	biopolymer coating
80445	Poly-D-Lysine, sterile*	biopolymer coating
80441	uncoated, sterile	hydrophobic
80447	glass bottom	glass coverslip No. 1.5H (170 μm ±5 μm)

\* available on request only

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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.