## 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 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-Plate 96 Well Black allows you to perform high resolution microscopy in a standard multi– well format. For less well–to–well crosstalk in fluorescence microscopy this imaging plate is made out of a black plastic material.

#### Material

ibidi  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates are made of a plastic that has the highest optical quality. The polymer coverslip on the bottom 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 Polymer Coverslip		
Refractive index n <sub>D</sub> (589 nm)	1.52	
Abbe number	56	
Thickness	No. 1.5 (180 μm)	
Material	Polymer coverslip	

Please note! The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 3.

### **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		
ibiTreat, Uncoated	36 months	

#### Geometry

The  $\mu$ -Plate 96 Well Black provides standard geometry and numbering (A-H, 1-12). The bottom of the  $\mu$ -Plate 96 Well Black provides a high accuracy.



The  $\mu$ -Plate 96 Well Black meets all important values of the ANSI/SLAS (SBS) Standards (1-2004, 2-2004, 3-2004 and 4-2004).

Dimensions of the µ-Pl	ate 96 Well Blac	k in mm
Length	127.8	$\pm 0.2$
Width	85.5	$\pm 0.2$
Height with lid	17.2	$\pm 0.4$
Height without lid	15.0	$\pm 0.4$
Well to well distance	9.0	$\pm 0.1$
Well clearance	0.8	$\pm 0.1$



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Single Well Parameters			
Single well dimensions	$7.4 \times 7.4 \pm 0.15 \text{ mm}$		
Single well depth	$12.9 \pm 0.2 \text{ mm}$		
Volume	300 µl		
Growth area	$0.56 \text{ cm}^2$		
Coating area using 300 µl	$2.35 \text{ cm}^2$		
Accuracy of the Bottom			
Inner well flatness	± 5 μm		
Whole plate flatness	$\pm 25 \mu m$		
Bottom matches coverslip	No. 1.5		

For manufacturing process reasons, the well wall angles differ slightly. The details are shown in the graphics below.



#### Surface

The  $\mu$ -Plate 96 Well Black is available with ibiTreat and uncoated surface. The ibiTreat surface is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested.

A specific coating of the  $\mu$ -Plate 96 Well Black can be done yourself following the procedure in the section Coating Your  $\mu$ -Plate 96 Well Black.

### Coating Your µ-Plate 96 Well Black

The hydrophobic, uncoated version of the  $\mu$ -Plate 96 Well Black 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 surface of the  $\mu$ -Plate 96 Well Black, 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  $300 \,\mu$ l in each well. The coating area using  $300 \,\mu$ l is  $2.35 \,\text{cm}^2$ .
- Follow your coating protocol.

Further information about coatings is provided in Application Note 08 "Cell culture coating".

## Remove the Protection Film Before Usage

The bottom of the  $\mu$ -Plate 96 Well Black is covered with a film to protect the optical quality of the plastic surface. Please pull off the protection film before usage!

## Seeding Cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a  $2-5 \times 10^4$  cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 300 µl cell suspension into each single well. Avoid shaking, as this will result in inhomogeneous distribution of the cells.
- Cover the  $\mu$ -Plate 96 Well Black with the supplied lid. Incubate at 37°C and 5% CO<sub>2</sub> as usual.



Undemanding cells can be left in their seeding medium for several days and grow to confluence there. However, best results might be achieved when the medium is changed every 2–3 days. Carefully aspirate the old medium and replace it by 300 µl fresh medium.

#### Tip:

You can stack the  $\mu$ -Plates to save space in you incubator. This will not affect cell growth. We recommend making batches with not more than 6 plates, due to stability reasons.

### Microscopy

To analyze your cells, no special preparations are necessary. Cells can be directly observed live or fixed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and storage of fixed and stained samples, ibidi provides a mounting medium (50001) optimized for  $\mu$ -Dishes,  $\mu$ - Slides, and  $\mu$ -Plates.

### **Chemical Compatibility**

The table below provides some basic information on the chemical and solvent compatibility of the  $\mu$ -Plate 96 Well Black. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on ibidi.com.

Chemical / Solvent	Compatibility
Methanol	yes
Ethanol	yes
Formaldehyde	yes
Acetone	yes, without lid
Mineral oil	no
Silicone oil	yes
Immersion oil	See <b>Immersion Oil</b> on page 3.

### **Immersion Oil**

When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non-recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are not listed in the table below should be considered as non-compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil	50101	16-12-27	01/2017
Zeiss	Immersol 518 F	444960	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012
Leica	Immersion Liquid	11513859	n.a.	03/2011
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017



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#### **Ordering Information**

The ibidi µ-Plates are available with the following surfaces.

#### μ-Plate 24 Well Black

Ca	at. No.	Description
82	2406	<b>μ-Plate 24 Well Black ibiTreat</b> : #1.5 polymer coverslip, tissue culture treated, sterilized
82	2401	<b>μ-Plate 24 Well Black Uncoated</b> : #1.5 polymer coverslip, hydrophobic, sterilized

#### µ-Plate 96 Well Black

Cat. No.	Description
89626 89621	<b>μ-Plate 96 Well Black ibiTreat</b> : #1.5 polymer coverslip, tissue culture treated, sterilized <b>μ-Plate 96 Well Black Uncoated</b> : #1.5 polymer coverslip, hydrophobic, sterilized
89621	<b>µ-riate 96 Weil Black Uncoated</b> : #1.5 polymer coverslip, hydrophobic, sterilized

#### μ-Plate 384 Well Clear



Cat. No.	Description
88416	<b>μ-Plate 384 Well Clear ibiTreat</b> : #1.5 polymer coverslip, tissue culture treated, sterilized
88411	$\mu$ -Plate 384 Well Clear Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized

#### 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, Lochhamer Schlag 11, 82166 Gräfelfing, Germany.