

#### Instructions

# Glass Bottom Dish 35 mm



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, super resolution and single molecule applications.

The Glass Bottom Dish 35 mm is a standard glass bottom dish for cell culture and high resolution microscopy.

#### **Material**

The Glass Bottom Dish  $^{35\,\mathrm{mm}}$  is made of a standard 35 mm Dish, but with a glass coverslip bottom. It is not possible to detach the bottom. The Dishes are not autoclavable since they are temperature stable only up to  $80^{\circ}\mathrm{C}$  /  $175^{\circ}\mathrm{F}$ .

### Optical Properties Glass Bottom Dish 35 mm

Refractive index  $n_D$  1.523 Abbe number 55 Thickness No. 1

No. 1.5

 $(170\,\mu m \, (+20\,\mu m/-10\,\mu m))$ 

Material Schott borosilicate glass,

D 263M

#### Attention!

Be cautious when handling ibidi labware products with glass bottom! The glass coverslip or glass slide is very fragile and might break easily. Handle with care to avoid physical injury and damage to devices through leakage of the medium.

# Geometry

Geometry of the Glass Bottom Dish 35 mm		
Diameter dish	35 mm	
Volume	2 ml	
Growth area	$3.14 \text{ cm}^2$	
Coating area using 400 µl	$3.7 \text{ cm}^2$	
Diameter observation area	20 mm	
Height with / without lid	11.7 mm/9.9 mm	
Bottom	Glass coverslip No. 1.5	

## **Surface and Coating**

The Glass Bottom Dish <sup>35 mm</sup> 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 Glass Bottom Dish <sup>35 mm</sup>. Adjust the concentration to a coating area of 3.7 cm<sup>2</sup> and a coating volume of 400 µl.
- Apply  $400\,\mu l$  into the growth area. Make sure that the entire bottom is covered with liquid easily tilting or shaking the  $\mu$ -Dish. Put on the lid and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash. Optionally, let dry at room temperature.

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

# **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 Storage conditions	Ambient RT (15-25°C)	
Shelf Life		
Glass Bottom	36 months	



### **Seeding Cells**

Depending on your cell type, application of a 4–9  $\times$   $10^4$  cells/ml suspension should result in a confluent layer within 2–3 days.

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration.
- Apply 400 μl cell suspension into the inner well of the μ–Dish. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- After cell attachment add additionally 1.6 ml of pure medium to ensure optimal grow conditions.
- Cover the  $\mu$ -Dish with the supplied lid. Incubate at 37°C and 5 % CO<sub>2</sub> as usual.

We recommend not to fill more than the indicated total volume into the Glass Bottom Dish <sup>35 mm</sup> in order to avoid the liquid contacting the lid.

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

#### **Glass Bottom Dish Selection Guide**

	Glass Bottom	μ–Dish <sup>35mm, high</sup>
	Dish 35 mm	Glass Bottom
Lid	normal	with Lid Lock position
Evaporation	medium	low
Coverslip	No. 1.5	No. 1.5H
Thickness	170 μm (+20 μm/-10 μm)	170 μm (±5 μm)
Packaging	10 pieces	1 piece



#### Note:

If cultivated in a dry atmosphere, evaporation can harm the cells during longer experiments. Exchange the medium every 1-2 days to minimize evaporationmediated osmolality shifts.

### **Optional Glass Coverslip Cleaning Protocol**

The Glass Bottom Dish <sup>35 mm</sup> is made with an uncoated glass coverslip. For improved cell attachment, glass surface modifications and other applications, the glass coverslip of the Glass Bottom Dish <sup>35 mm</sup> can be cleaned by following the protocol below.

- Remove lids and immerse products in ddH<sub>2</sub>O in an appropriately sized beaker.
- Sonicate for 10 minutes.
- Decant the ddH<sub>2</sub>O completely.
- Add 1 M HCl.
- Sonicate for 10 minutes.
- Decant the HCl completely and wash twice with ddH<sub>2</sub>O. Decant the ddH<sub>2</sub>O completely.
- Add 2-propanol (absolute).
- Sonicate for 10 minutes.
- Aspirate the 2-propanol completely. Make sure that all products are completely dry. Wash twice with ddH<sub>2</sub>O and aspirate the ddH<sub>2</sub>O completely.
- Add ethanol (absolute).
- Sonicate for 10 minutes.
- Aspirate the ethanol completely. Make sure that all products are completely dry. Wash twice with ddH<sub>2</sub>O.
- Sonicate in ddH<sub>2</sub>O for 10 min.
- Decant ddH<sub>2</sub>O and blow dry carefully with canned air or clean nitrogen gas.

Modifications of this protocol including acids, bases, alcohols and detergents are possible. Please check the chemical compatibility list on <a href="www.ibidi.com">www.ibidi.com</a> for compatibility. Make sure to handle the glass-bottomed products with care. The glass coverslips may break during mechanical handling. For best results, use a custom-made Teflon holder.

# Glass Bottom Dish 35 mm

### Instructions

### **Cell Microscopy and Solvents for Fixation**

To analyze your cells, no special preparations are necessary. Cells can be observed live, or fixed directly in the Glass Bottom Dish  $^{35\,\mathrm{mm}}$ , preferably on an inverted microscope. Due to the thin bottom, high resolution microscopy

is possible. The material is compatible to most fixatives, like acidic acid, alcohols and PFA. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on www.ibidi.com. For optimal results in fluorescence microscopy and storage of stained probes ibidi provides a mounting medium (50001) optimized for  $\mu\text{-Dishes}$  and  $\mu\text{-Slides}.$ 

### **Ordering Information**

# $\mu$ –Dish $^{35 \text{ mm, high}}$



Cat. No.	Description
81156	$\mu$ –Dish <sup>35 mm, high</sup> ibiTreat: $\varnothing$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized
81156-400	$\mu$ –Dish <sup>35 mm, high</sup> ibiTreat, Bulk Pack: $\emptyset$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized
81151	$\mu$ –Dish <sup>35 mm, high</sup> Uncoated: $\varnothing$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, hydrophobic, sterilized

### μ-Dish <sup>35 mm, high</sup> Grid-500



Cat. No.	Description
81166	μ–Dish <sup>35 mm, high</sup> Grid-500 ibiTreat: Ø 35 mm, high wall (2 ml volume), grid repeat distance
81161	500 μm, #1.5 polymer coverslip, tissue culture treated, sterilized μ–Dish <sup>35 mm, high</sup> Grid-500 Uncoated: Ø 35 mm, high wall (2 ml volume), grid repeat dis-
01101	tance 500 µm,#1.5 polymer coverslip, hydrophobic, sterilized

### Glass Bottom Dish 35 mm



Cat. No.	Description
81218-200	Glass Bottom Dish $^{35\text{mm}}$ : $\varnothing$ 35 mm, high wall (2 ml volume), #1.5 (170 $\mu$ m (+20 $\mu$ m/-10 $\mu$ m)) D 263 M Schott glass, sterilized, 200 pieces
81218-800	Glass Bottom Dish $^{35\mathrm{mm}}$ : $\varnothing$ 35 mm, high wall (2 ml volume), #1.5 (170 $\mu$ m (+20 $\mu$ m/-10 $\mu$ m)) D 263 M Schott glass, sterilized, 800 pieces

### μ-Dish 35 mm, high Glass Bottom



Cat. No.	Description
81158	$\mu$ –Dish <sup>35 mm, high</sup> Glass Bottom: Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized
81158-400	<b>μ–Dish</b> <sup>35 mm, high</sup> <b>Glass Bottom, Bulk Pack</b> : Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized



# Glass Bottom Dish 35 mm

# Instructions

μ-Dish <sup>35 mm, low</sup>



Cat. No.	Description
80136	$\mu$ –Dish <sup>35 mm, low</sup> ibiTreat: Ø 35 mm, low wall (800 $\mu$ l volume), #1.5 polymer coverslip, tissue
80131	culture treated, sterilized μ–Dish <sup>35 mm, low</sup> Uncoated: Ø 35 mm, low wall (800 μl volume), #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, Am Klopferspitz 19, 82152 Martinsried, Germany.