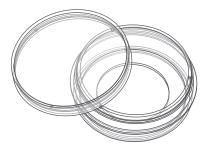


# μ-Dish<sup>35 mm, high</sup> Glass Bottom

Instruction Manual



The ibidi labware is comprised of a variety of  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates, which have all been designed for high-end microscopic analysis of fixed or living cells. The glass bottom versions are especially designed for TIRF, superresolution, and single molecule applications. The  $\mu$ -Dish <sup>35 mm, high</sup> Glass Bottom allows you to perform high-resolution microscopy in a 35 mm Petri dish with 12 mm walls. The standard height allows convenient liquid handling. The lid can be closed to hinder evaporation during long-term experiments.

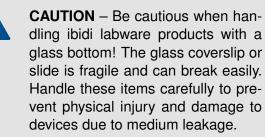
This document applies to the following product:

81158 µ-Dish <sup>35 mm, high</sup> Glass Bottom

# Material

The  $\mu$ -Dish <sup>35 mm, high</sup> Glass Bottom is made with a glass coverslip bottom. It is not possible to detach the bottom from the upper part. The dish is intended for one-time use and is not autoclavable, since it is only temperature-stable up to 80 °C/175 °F.

<b>Optical Properties of Glass Coverslip</b>		
Refractive index	1.523	
Abbe number	55	
Thickness	No. 1.5H (170 μm ± 5 μm)	
Material	Schott borosilicate glass, D 263 M	



### Shipping and Storage

This product is sterilized and sealed in a gaspermeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

Conditions			
Shipping conditions	Ambient		
Storage conditions	RT (15–25℃)		
Shelf Life			

Shelf Life		
Glass Bottom	36 months	

# Geometry

Specifications			
ø μ-Dish	35 mm		
Volume	2 ml		
Growth area	3.5 cm <sup>2</sup>		
Coating area	4.1 cm <sup>2</sup>		
Ø observation area	21 mm		
Height with / without lid	14/12 mm		
Bottom	Glass coverslip No. 1.5H		

### Surface

The  $\mu$ -Dish<sup>35 mm, high</sup> Glass Bottom is manufactured with a glass coverslip. Washing it (e.g., with PBS) before cell seeding helps removing glass dusts, which enhances direct cell growth on the surface.

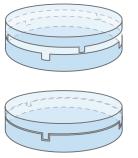
# Coating

Detailed information about coatings is provided in Application Note 08: Coating Protocols for ibidi Labware.

In short, specific coatings are possible following this protocol:

- Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 4.1 cm<sup>2</sup> and a volume of 400 μl.
- Apply 400 µl into the central growth area. Make sure that the entire bottom of the dish is covered with liquid by gently tilting or shaking it. Close the lid and leave the dish at room temperature for at least 30 minutes.
- 3. Aspirate the solution and wash with the recommended protein dilution buffer.
- 4. The coated dish is ready to be used. Be aware that allowing the dish to dry out is not recommended, as some coating proteins may degrade upon drying.

# Lid with Locking Feature for Minimized Evaporation



Open position for easy opening

Closed position for cell cultivation with minimal evaporation



Lock position for long-term studies with almost no evaporation

# Seeding Cells

- 1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. 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.
- 2. Apply 400 μl cell suspension into the growth area of the dish. Avoid shaking, as this will result in inhomogeneous cell distribution.
- 3. After cell attachment, add 1.6 ml of medium to ensure optimal growing conditions.
- 4. Cover the dish with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO<sub>2</sub>).

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**CAUTION** – We do not recommend filling more than 2 ml into the  $\mu$ -Dish<sup>35 mm, high</sup> Glass Bottom in order to avoid the liquid contacting the lid.

Insensitive cells can be left in their seeding medium for several days and grow to confluence there. However, optimal results might be achieved when the medium is changed every 2–3 days. For this, carefully aspirate the old medium and replace it by up to 2 ml fresh medium.



**TIP** – You can stack the  $\mu$ -Dishes to save space in your incubator. This will not affect cell growth. Due to stability reasons, we recommend making batches with not more than 6  $\mu$ -Dishes. Placing the  $\mu$ -Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination each time the incubator is opened.

# **Optional: Glass Coverslip Cleaning**

The  $\mu$ -Dish <sup>35 mm, high</sup> Glass Bottom is made with an uncoated glass coverslip. For special applications, the coverslip of the dish can be cleaned by following the protocol below.

- 1. Remove lids and immerse the dish in ddH<sub>2</sub>O in an appropriately sized beaker.
- 2. Sonicate for 10 minutes.
- 3. Decant the  $ddH_2O$  completely.
- 4. Add 1 M HCl and sonicate for 10 minutes.
- 5. Decant the HCl completely and wash twice with ddH<sub>2</sub>O. Decant the ddH<sub>2</sub>O completely.
- 6. Add absolute 2-propanol and 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.
- 8. Add absolute ethanol and sonicate for 10 minutes.
- Aspirate the ethanol completely. Make sure that all products are completely dry. Wash twice with ddH<sub>2</sub>O.
- 10. Sonicate in  $ddH_2O$  for 10 min.
- 11. 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 Section "Chemical Compatibility". Make sure to handle the glass-bottomed products with care. The glass coverslips may break during mechanical handling. For optimal results, use a custom-made teflon holder.

#### Microscopy

To image your cells, no special preparations are necessary. Living or fixed cells can be directly observed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and for storage of fixed and stained samples, ibidi provides mounting media that are optimized for ibidi labware:

Cat. No. 50001: ibidi Mounting Medium Cat. No. 50011: ibidi Mounting Medium with DAPI

#### **Immersion Oil**

When using ibidi Glass Bottom products with oil immersion objectives, there is no known incompatibility with any immersion oil on the market. All types of immersion oils can be used.

#### **Chemical Compatibility**

The following table provides some basic information on the chemical and solvent compatibility of the  $\mu$ -Dish<sup>35 mm, high</sup> Glass Bottom. For a full list of compatible solvents and more information on chemical compatibility, visit ibidi.com/chemicals.

<b>Chemical / Solvent</b>	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	Yes, without lid
Mineral oil	Yes
Silicone oil	Yes
Immersion oil	See Section "Immer- sion Oil"

#### For research use only!

Further information can be found at ibidi.com. For questions and suggestions, please contact us by e-mail at info@ibidi.com or by telephone at +49 (0)89/520 4617 0. © ibidi GmbH, Lochhamer Schlag 11, 82166 Gräfelfing, Germany.