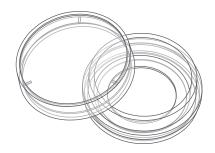
# Glass Bottom Dish 35 mm

Instruction Manual



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

This document applies to the following products:

81218-200 Glass Bottom Dish <sup>35 mm</sup> 81218-800 Glass Bottom Dish <sup>35 mm</sup>

#### **Material**

The Glass Bottom Dish <sup>35 mm</sup> is made of a standard 35 mm Dish, but with a glass coverslip bottom. Also, 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.

Optical Properties	s of Glass Coverslip
Refractive index	1.523
Abbe number	55
Thickness	No. 1.5,
	170 μm (+20/-10 μm)
Material	Schott borosilicate
	glass, D 263 M



**CAUTION** – Be cautious when handling ibidi labware products with a glass bottom! The glass coverslip or slide is fragile and can break easily. Handle these items carefully to prevent physical injury and damage to devices due to medium leakage.

#### **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		
Glass Bottom	36 months	

## Geometry

Specifications		
ø Dish	35 mm	
Volume	2 ml	
Growth area	3.14 cm <sup>2</sup>	
Coating area	$3.7\mathrm{cm}^2$	
ø observation area	20 mm	
Height with / without lid	11.7 mm / 9.9 mm	
Bottom	Glass coverslip No. 1.5H	

#### **Surface**

The Glass Bottom Dish <sup>35 mm</sup> 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.

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## 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 3.7 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.

#### **Seeding Cells**

- Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 5–11 x 10<sup>4</sup> 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.
- 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 ℃ and 5% CO<sub>2</sub>).



**CAUTION** – We do not recommend filling more than 2 ml into the Glass Bottom Dish <sup>35 mm</sup> 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.



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

## **Optional: Glass Coverslip Cleaning**

The Glass Bottom Dish <sup>35 mm</sup> 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<sub>2</sub>O 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<sub>2</sub>O 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 Glass Bottom Dish <sup>35 mm</sup>. For a full list of compatible solvents and more information on chemical compatibility, visit ibidi.com/chemicals.

Chemical / Solvent	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	Yes, without lid
Mineral oil	Yes
Silicone oil	Yes
Immersion oil	See Section "Immersion 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.

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