

# u-Dish <sup>35 mm, low</sup> Glass Bottom

#### 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, super resolution and single molecule applications. The  $\mu$ -Dish  $^{35\,\text{mm,low}}$  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.

The  $\mu$ -Dish  $^{35 \text{ mm, low}}$  allows you to perform high resolution microscopy in a 35 mm Petri–dish with 7 mm walls. The low height makes high numerical apertures of Köhler illumination possible and provides large access for micromanipulation. The lid can be closed to hinder evaporation during long term experiments.

#### **Material**

The  $\mu$ -Dish  $^{35\,\text{mm,low}}$  Glass Bottom is made with a glass coverslip bottom. It is not possible to detach the bottom. The  $\mu$ -Dish  $^{35\,\text{mm,low}}$  Glass Bottom is intended for one-time use and not autoclavable since it is temperature stable only up to  $80^{\circ}\text{C}/175^{\circ}\text{F}$ .

#### Optical Properties of the Glass Coverslip Bottom

Refractive index n <sub>D</sub>	1.523
Abbe number	55
Thickness	No. 1.5H (selected quality 170 $\mu$ m, $\pm$ 5 $\mu$ m)
Material	Schott borosilicate glass, D 263M

#### **Surface and Coating**

The  $\mu$ -Dish  $^{35\,\text{mm,low}}$  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  $\mu$ -Dish  $^{35\,\mathrm{mm,low}}$  Glass Bottom. Adjust the concentration to a coating area of  $4.1\,\mathrm{cm^2}$  and a coating volume of  $400\,\mu$ l.
- Apply 400 μl into the growth area. Make sure that the entire bottom is covered with liquid easily tilting or shaking the μ-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".

### Geometry

Geometry of the μ-Dish	35 mm, low Glass Bottom
Diameter dish	35 mm
Volume	0.8 ml
Growth area	$3.5 \text{ cm}^2$
Coating area using 400 µl	$4.1 \text{ cm}^2$
Diameter observation area	21 mm
Height with / without lid	9 mm / 7 mm
Bottom	Glass coverslip No. 1.5H

#### **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



#### 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.

### **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 400 µl 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 do not recommend filling more than 800  $\mu$ l into the  $\mu$ -Dish  $^{35\,\text{mm,low}}$  Glass Bottom 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  $800~\mu$ l fresh medium.

## **Optional Glass Coverslip Cleaning Protocol**

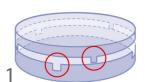
The  $\mu$ -Dish  $^{35\,\text{mm,low}}$  Glass Bottom is made with an uncoated glass coverslip. For special applications, the coverslip of the  $\mu$ -Dish  $^{35\,\text{mm,low}}$  Glass Bottom can be cleaned by following the protocol below.

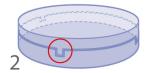
- Remove lids and immerse products in ddH<sub>2</sub>O in an appropriately sized beaker.
- Sonicate for 10 minutes.
- Decant the ddH<sub>2</sub>O completely.
- Add 1 M HCl.
- Sonicate for 10 minutes.

- 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.
- ullet 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="https://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.

#### **Using The Lid**





- 1. Open position, easy opening
- 2. Close position, for long term studies, minimal evaporation

#### **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.



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## **Chemical Compatibility**

The following table provides some basic information on the chemical and solvent compatibility of the  $\mu$ -Dish  $^{35\,\mathrm{mm,low}}$  Glass Bottom. 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	yes
Silicone oil	yes
Immersion oil	See <b>Immersion Oil</b> on page 2.

#### **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  $\mu\text{-Dishes}$ ,  $\mu\text{-Slides}$ , and  $\mu\text{-Plates}$ .

### **Minimizing Evaporation**

Using the  $\mu$ -Dish with a closed lid, the evaporation in an incubator system with 37°C and 95% humidity is around 1% per day. Using the  $\mu$ -Dish with a closed lid in a 37°C heating system with low humidity (between 20% and 40%), the evaporation is around 10% per day. For reducing the evaporation down to 1% per day in all systems, we recommend sealing the lid with ibidi Anti-Evaporation Oil (50051).

## Tip:

You can stack the  $\mu$ -Dishes to save space in your incubator. This will not affect cell growth. We recommend making batches with up to 6  $\mu$ -Dishes, due to stability reasons. Placing the  $\mu$ -Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination when the incubator is opened.

## **Instructions**

# $\mu\text{-Dish}^{\,35\,mm,\,low}$ Glass Bottom

## **Ordering Information**

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



Cat. No.	Description
81156	$\mu$ -Dish $^{35 \text{ mm, high}}$ ibiTreat: $\emptyset$ 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	<b>μ-Dish</b> <sup>35 mm, high</sup> <b>Uncoated</b> : Ø 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, hydrophobic, sterilized
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	$\mu$ -Dish <sup>35 mm, high</sup> Glass Bottom, Bulk Pack: Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized

#### u-Dish 35 mm, high 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 distance
	500 μm,#1.5 polymer coverslip, hydrophobic, sterilized

## Glass Bottom Dish 35 mm



Cat. No.	Description
81218-200	Glass Bottom Dish <sup>35 mm</sup> : Ø 35 mm, high wall (2 ml volume), #1.5 (170 μm (+20 μm/-10 μm)) D 263 M Schott glass, sterilized, 200 pieces
81218-800	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, 800 pieces

# 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 culture treated, sterilized
80131	$\mu$ -Dish <sup>35 mm, low</sup> Uncoated: Ø 35 mm, low wall (800 $\mu$ l volume), #1.5 polymer coverslip, hydrophobic, sterilized
80137	$\mu$ -Dish <sup>35 mm, low</sup> Glass Bottom: Ø 35 mm, low wall (800 μl volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized

## For research use only!

Further information can be found at ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0.

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