Instructions





The ibidi product family is comprised of a variety of μ -Slides and μ -Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells.

The glass bottom versions of the μ -Slides and μ -Dishes are especially designed for TIRF, super resolution and single molecule applications. The μ -Dish ^{35 mm, high} 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.

Material

The μ -Dish^{35 mm, high} Glass Bottom is made with a glass coverslip bottom. It is not possible to detach the bottom. The μ -Dish^{35 mm, high} Glass Bottom is not autoclavable since it is temperature stable only up to 80°C/175°F.

| Optical Properties ibidi Glass Bottom | | |
|--|---|--|
| Refractive index n_D | 1.523 | |
| Abbe number | 55 | |
| Thickness | No. 1.5H (selected quality 170 μm, ± 5 μm) | |
| 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 µ–Dish | ^{35 mm, high} Glass Bottom |
|---------------------------|-------------------------------------|
| Diameter dish | 35 mm |
| Volume | 2 ml |
| Growth area | 3.5 cm^2 |
| Coating area using 400 µl | 4.1 cm^2 |
| Diameter observation area | 21 mm |
| Height with / without lid | 14 mm / 12 mm |
| Bottom | Glass coverslip No. 1.5H |

Surface and Coating

The μ -Dish^{35 mm, high} 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 μ -Dish^{35 mm, high} Glass Bottom. Adjust the concentration to a coating area of 4.1 cm² and a coating volume of 400 μ 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".

Shipping and Storage

The μ -Slides, μ -Dishes and μ -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 | | |
| Glass Bottom | 36 months | | |
| | | | |

μ-Dish^{35 mm, high} Glass Bottom

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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 μ -Dish with the supplied lid. Incubate at 37°C and 5 % CO₂ as usual.

We recommend not to fill more than the indicated total volume into the μ -Dish^{35 mm, high} 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 2 ml fresh medium.

Optional Glass Coverslip Cleaning Protocol

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

- Remove lids and immerse products in ddH₂O in an appropriately sized beaker.
- Sonicate for 10 minutes.
- Decant the ddH₂O completely.
- Add 1 M HCl.
- Sonicate for 10 minutes.
- Decant the HCl completely and wash twice with ddH₂O. Decant the ddH₂O 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 ddH₂O and aspirate the ddH₂O completely.
- Add ethanol (absolute).
- Sonicate for 10 minutes.

- Aspirate the ethanol completely. Make sure that all products are completely dry. Wash twice with ddH_2O .
- Sonicate in ddH₂O for 10 min.
- Decant ddH₂O 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 www.ibidi.com 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.

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 μ -Dishes, μ -Slides, and μ -Plates.



Minimizing Evaporation

Using the μ -Dish with a closed lid, the evaporation in an incubator system with 37°C and 95% humidity is around 1% per day. Using the μ -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 μ -Dishes to save space in your incubator. This will not affect cell growth. We recommend making batches with up to 6 μ -Dishes, due to stability reasons. Placing the μ -Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination when the incubator is opened.

Chemical Compatibility

The table below provides some basic information on the chemical and solvent compatibility of the μ -Dish ^{35 mm, high} 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 Immersion Oil on page 2. |

Ordering Information

µ-Dish ^{35 mm, high}

| Cat. No. | Description |
|-----------|---|
| 81156 | μ-Dish ^{35 mm, high} ibiTreat : Ø 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized |
| 81156-400 | μ-Dish ^{35 mm, high} ibiTreat, Bulk Pack : \emptyset 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized |
| 81151 | μ-Dish ^{35 mm, high} Uncoated : \emptyset 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, hydrophobic, sterilized |

μ-Dish ^{35 mm, high} Grid-500

| Cat. No. | Description |
|----------|---|
| 81166 | μ-Dish ^{35 mm, high} Grid-500 ibiTreat : Ø 35 mm, high wall (2 ml volume), grid repeat distance 500 μm, #1.5 polymer coverslip, tissue culture treated, sterilized |
| 81161 | μ -Dish ^{35 mm, high} Grid-500 Uncoated: Ø 35 mm, high wall (2 ml volume), grid repeat distance 500 μ m,#1.5 polymer coverslip, hydrophobic, sterilized |



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Glass Bottom Dish ^{35 mm}

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

μ-Dish ^{35 mm, high} Glass Bottom

| Cat. No. | Description |
|-----------|---|
| 81158 | μ-Dish ^{35 mm, high} Glass Bottom : Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized |
| 81158-400 | μ -Dish ^{35 mm, high} Glass Bottom, Bulk Pack: \emptyset 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μ m) D 263 M Schott glass, sterilized |

μ -Dish ^{35 mm, low}

| Cat. No. | Description |
|-----------|---|
| 80136 | μ-Dish ^{35 mm, low} ibiTreat : Ø 35 mm, low wall (800 μl volume), #1.5 polymer coverslip, tissue culture treated, sterilized |
| 80131 | $\mu\text{-Dish}~^{35\text{ mm, low}}$ Uncoated: ø 35 mm, low wall (800 μl volume), #1.5 polymer coverslip, hydrophobic, sterilized |

For research use only!

Further information 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.

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