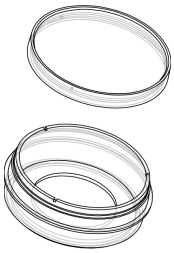


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells.
The glass bottom versions of the µ–Slides and µ–Dishes are especially designed for TIRF and single molecule applications. The µ–Dish 35mm, 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 35mm, high Glass Bottom is made of a standard µ–Dish 35mm, high but with a glass coverslip bottom. It is not possible to detach the bottom. The µ–Dishes are not autoclavable since they are 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 µ–Slides and µ–Dishes with glass bottom! The glass coverslip 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 35mm, high Glass Bottom	
Diameter dish	35 mm
Volume	2000 µl
Growth area	3.5 cm^2
Diameter growth area	21 mm
Coating area using 400 µl	4.2 cm^2
Height with / without lid	14 mm / 12 mm
Bottom	Glass coverslip No. 1.5H

Surface and Coating

The µ–Dish 35mm, 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. Adjust the concentration to a coating area of 4.2 cm^2 and 400 µl.
- 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.

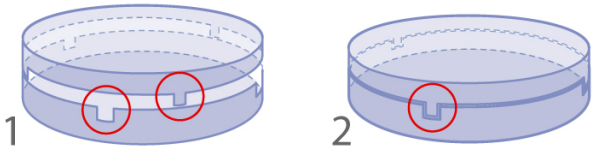
Detailed information about coatings is provided in Application Note 08 "Cell culture coating".

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 of Different Surfaces	
ibiTreat, Glass Bottom, ESS	36 months
Collagen, Poly-Lysine	18 months
Fibronectin	4 months

Using The Lid



1. open position, easy opening
2. close position, for long term studies, minimal evaporation

Seeding Cells

Depending on your cell type, application of a 4–9 × 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 % CO_2 as usual.

We recommend not to fill more than the indicated total volume into the µ–Dish 35mm, 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.

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.

Preparation for Cell Microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Dish preferably on an inverted microscope. You can use any fixative of your choice. The µ–Dish material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom high resolution microscopy is possible.

For optimal results in fluorescence microscopy and storage of stained probes, ibidi provides a mounting medium optimized for µ–Dishes and µ–Slides (ibidi Mounting Medium, 50001).

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).

Immersion Oil

When using oil immersion objectives, use only the immersion oils specified in the table. The use of a non–recommended oil could lead to the damage of the plastic material and the objective.

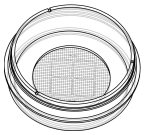
Company	Product	Ordering Number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

µ–Dish 35mm, high Family

µ–Dish 35mm, high

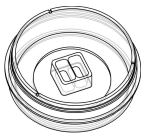
Cat. No.	Description	Characteristics
81156	**µ–Dish 35mm, high ibiTreat**: ⌀ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated	hydrophilic, sterilized
81151	**µ–Dish 35mm, high Uncoated**: ⌀ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip	hydrophobic, sterilized

µ–Dish 35mm, high Grid–500



Cat. No.	Description	Characteristics
81166	**µ–Dish 35mm, high ibiTreat Grid-500**: ⌀ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, grid repeat distance 500 µm	hydrophilic, sterilized
81161	**µ–Dish 35mm, high Uncoated Grid-500**: ⌀ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, grid repeat distance 500 µm	hydrophobic, sterilized

Culture–Insert in µ–Dish 35mm, high



Cat. No.	Description	Characteristics
81176	**Culture–Insert in µ–Dish 35mm, high ibiTreat**: ready to use, tissue culture treated	hydrophilic, sterilized

micro–Insert 4 Well in µ–Dish 35mm, high

Cat. No.	Description	Characteristics
80406	**micro–Insert 4 Well in µ–Dish 35mm, high ibiTreat**: ready to use, tissue culture treated	hydrophilic, sterilized

µ–Dish 35mm, high Glass Bottom

Cat. No.	Description	Characteristics
81158	**µ–Dish 35mm, high Glass Bottom**: ⌀ 35 mm, high wall (2 ml volume), #1.5H (170 ±5 µm) D 263 M Schott glass	sterilized

µ–Dish 35mm, high ESS

Cat. No.	Description	Characteristics
81291	**µ–Dish 35mm, high ESS 1.5 kPa Uncoated**: ⌀ 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 1.5 kPa	hydrophobic, sterilized
81391	**µ–Dish 35mm, high ESS 15 kPa Uncoated**: ⌀ 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 15 kPa	hydrophobic, sterilized
81191	**µ–Dish 35mm, high ESS 28 kPa Uncoated**: ⌀ 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 28 kPa	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.