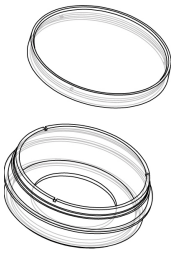


The ibidi product family comprises a variety of different shapes of µ–Slides, µ–Dishes and plates which all have been designed for high-end microscopic analysis of fixed or living cells.

The glass bottom version of µ–Dish 35 mm, high is especially designed for TIRF and single molecule applications. It 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

µ–Dish 35 mm, high glass bottom consists of a standard µ–Dish 35 mm, 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 60 °C / 140°F.

Optical properties ibidi glass bottom	
Refractive index n_D	1.523
Abbe number	55
Thickness	No. 1.5 (selected quality 170 µm, ± 10 µm)
Material	Schott borosilicate glass, D 263M

Geometry

Geometry of the µ–Dish 35 mm, 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

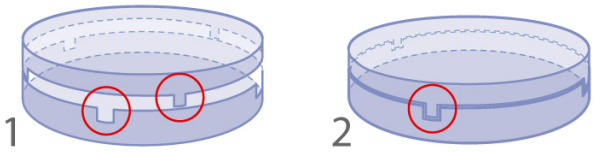
Surface and coating

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. 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.

Using the lid



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

Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. 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.
- 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 2 ml into the µ–Dish 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 might be 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.

For optimal results in fluorescence microscopy and storage of stained probes ibidi provides a mounting medium optimized for µ–Dishes and µ–Slides.

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 silicon oil AR 200.

Immersion oil

When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the plastic material and the objective.

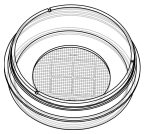
Company	Product	Ordering number
Cargille	type DF, Code: 1261	(Cargille) 16242
Cargille	type DF37, Code: 0383	(Cargille) 16239
Cargille	Series AAA, n = 1.330	(Cargille)
Cargille	Series AAA, n = 1.335	(Cargille)
ibidi	immersion oil	(ibidi) 50101
Olympus	8 ml	(Olympus) 035520
Zeiss	518 F	(Zeiss) 444960

µ–Dish 35 mm, high family

µ–Dish 35 mm, high

Ordering number	Treatment or Coating	Characteristics
81156	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
81151	uncoated, sterile	hydrophobic

µ–Dish 35 mm, high with Grid-500



Ordering number	Treatment or Coating	Characteristics
81166	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
81161	uncoated, sterile	hydrophobic

µ–Dish 35 mm, high with Culture-Insert

Ordering number	Treatment or Coating	Characteristics
81176	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
81171	uncoated, sterile	hydrophobic

µ–Dish 35 mm, high with Culture-Insert StemCell

Ordering number	Treatment or Coating	Characteristics
80406	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80401	uncoated, sterile	hydrophobic

µ–Dish 35 mm, high glass bottom

Ordering number	Treatment or Coating	Characteristics
81158	glass bottom, sterile	uncoated glass coverslip

µ–Dish 35 mm, high with ESS

Ordering number	Treatment or Coating	characteristics
81191	elastic surface ESS, 28 kPa, uncoated, sterile	hydrophobic
81192	elastic surface ESS, 28 kPa, coated Collagen IV, sterile	protein coating

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.