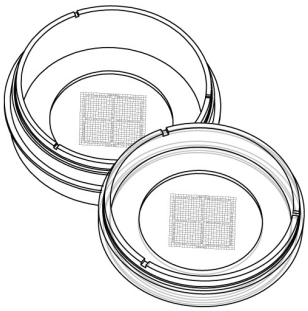


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

The Grid–500 is a grid structure for relocating events. It provides 400 distinguishable observation squares of 500 µm edge length. The grid is clearly visible by phase contrast microscopy and based on the high quality ibidi Standard Bottom. The outer dimensions are identical to ibidi µ–Dishes.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates are made of a plastic that has the highest optical quality. The bottom material exhibits extremely low birefringence and autofluorescence, similar to that of glass. Also, it is not possible to detach the bottom from the upper part. The µ–Slides, µ–Dishes, and µ–Plates are not autoclavable, since they are only temperature–stable up to 80°C/175°F. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the polymer coverslip, which should not be covered.

Optical Properties ibidi Standard Bottom	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	microscopy plastic/ polymer coverslip

Please note! The ibidi Standard Bottom is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 3.

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.

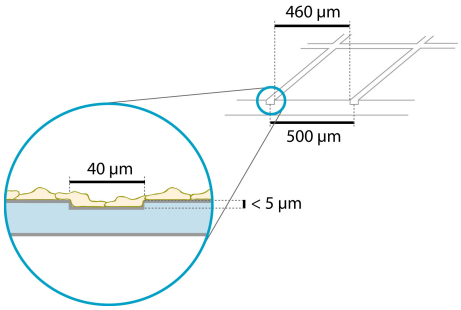
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15-25°C)

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

Characteristics of the Grid

The Grid–500 is made of small grooves inside the ibidi plastic surface of ibidi µ–Dishes. The structure is imprinted on the side on which cells are growing and does not effect cell growth, coating protocols, or surface properties. Proliferation and cell behavior is comparable with standard non–gridded dishes. Cells and grid are in one focal plane.

The grid is made of grooves, which are 40 µm (± 5 µm) wide and approximately 5 µm deep. Cells can grow in the grooves as well. We recommend using objective lenses up to 20 ×. Anyhow, the optical quality meets the requirements of 63 × and 100 × oil objective lenses as well (ibidi Standard Bottom, No. 1.5).

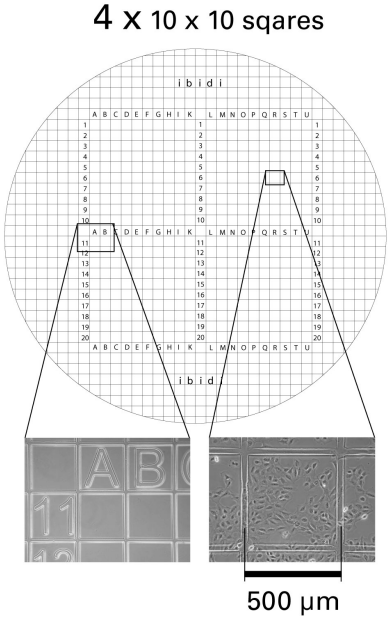


Geometry of the Grid–500

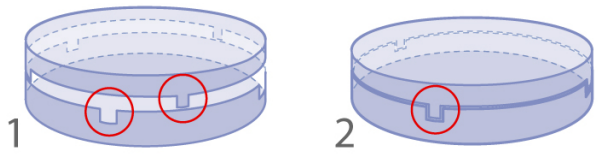
Geometry of the Grid–500	
Number of squares	400
Repeat distance	500 µm
Groove width	40 µm (± 5 µm)
Groove depth	< 5 µm

The four major squares are separated in 10 × 10 observation fields and indicated by letters and numbers ranging from:

- A to K (J not used) and 1 to 10
- A to K (J not used) and 11 to 20
- L to U and 1 to 10
- L to U and 11 to 20



Using The Lid



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

Surface and Coating

The µ–Dish 35mm Grid-500 is available with ibiTreat and uncoated surface. The ibiTreat surface is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested for good cell growth. The uncoated surface is a very hydrophobic surface and allows no direct cell growth. It is suitable for specific coatings or suspension cells.

If you like to establish a particular coating for your demands we recommend to test your coating procedure on uncoated and ibiTreat µ–Dishes, since some biomolecules adhere differently to hydrophobic or hydrophilic plastic surfaces.

- Prepare your coating solution according to the manufacturer's specifications or reference. Prepare your µ–Dish, ibiTreat or uncoated. 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".

The µ–Dish 35mm, high is also available with an elastically supported surface (ESS) and a glass bottom surface. Please refer to the instructions for detailed information.

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 (400 µl for the µ–Dish 35mm with low walls) 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 Grid-500 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 (800 µl for the µ–Dish 35mm with low walls) 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.

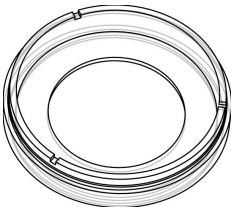
For optimal results in fluorescence microscopy and storage of stained probes, ibidi provides a mounting medium optimized for µ–Dishes and µ–Slides (ibidi Mounting Medium, 50001).

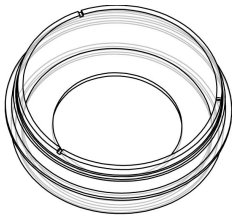
Minimizing Evaporation

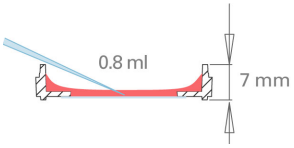
Using the µ–Dish with a closed lid, the evaporation in an incubator system with 37°C and 95 % humidity is around 1 % per day. Using the µ–Dish with a closed lid in a 37°C heating system with low humidity (between 20 % and 40 %), the evaporation is around 10 % per day. For reducing the evaporation down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

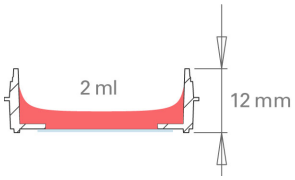
µ–Dish 35mm Selection Guide

µ–Dish 35mm, low	µ–Dish 35mm, high
Low walls (7 mm) for large access to the cells. Designed for micromanipulation and microinjection.	High walls (12 mm) for all standard applications. Also available with glass bottom, relocation grid, and elastic surface (ESS).









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

Selected References

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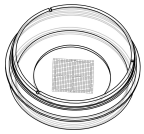
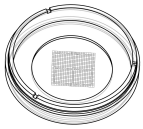
µ–Dish 35mm Grid Family

µ–Dish 35mm, high Grid-500



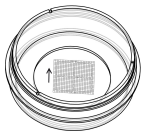
Cat. No.	Description	Characteristics
81166	**µ–Dish 35mm, high ibiTreat Grid-500**: ⌀ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, grid repeat distance 500 µm	hydrophilic, sterilized
81161	**µ–Dish 35mm, high Uncoated Grid-500**: ⌀ 35 mm, high wall (2 ml volume), #1.5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

µ–Dish 35mm, low Grid-500



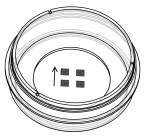
Cat. No.	Description	Characteristics
80156	**µ–Dish 35mm, low ibiTreat Grid-500**: ⌀ 35 mm, high wall (800 µl volume), #1.5 polymer coverslip, tissue culture treated, grid repeat distance 500 µm	hydrophilic, sterilized
80151	**µ–Dish 35mm, low Uncoated Grid-500**: ⌀ 35 mm, high wall (800 µl volume), #1.5 polymer coverslip, grid repeat distance 500 µm	hydrophobic, sterilized

µ–Dish 35mm, high Glass Bottom Grid–500



Cat. No.	Description	Characteristics
81168	**µ–Dish 35mm, high Glass Bottom Grid–500**: ⌀ 35 mm, high wall (2 ml volume), #1.5H (170 ±5 µm) D 263 M Schott glass, grid repeat distance 500 µm	sterilized

µ–Dish 35mm, high Glass Bottom Grid–50



Cat. No.	Description	Characteristics
81148	**µ–Dish 35mm, high Glass Bottom Grid–50**: ⌀ 35 mm, high wall (2 ml volume), #1.5H (170 ±5 µm) D 263 M Schott glass, grid repeat distance 50 µm	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.