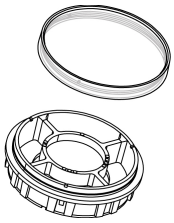


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ–Dish 35mm Quad allows you to perform high resolution microscopy in a 35 mm Petri-dish with a 4 well subdivision. The Ph+ structure in the center provides excellent phase contrast without a distracting meniscus. The lid can be closed to hinder evaporation during long term experiments.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates are made of a plastic that has the highest optical quality. The polymer coverslip on the bottom 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 Polymer Coverslip	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	polymer coverslip

Please note! The ibidi Polymer Coverslip 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.

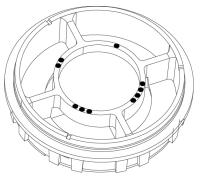
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15-25°C)

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

Geometry

Geometry of the µ–Dish 35mm Quad	
Diameter	35 mm
Volume per well	300 µl
Liquid height	4.0 mm
Growth area per well	0.85 cm^2
Coating area per well	2.46 cm^2
Partition wall	0.6 mm
Diameter growth area	21 mm
Height with/without lid	12 mm/10 mm
Bottom matches coverslip	No. 1.5

Identification of the wells



The wells are labelled by small points on the edge of the small plate to guarantee a definite identification.

Surface

The tissue culture treated ibiTreat surface is a physical surface modification and optimized for adhesion of most cell types. 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 testing your coating procedure on uncoated and ibiTreat surfaces, since some proteins and biomolecules adhere differently to hydrophobic or hydrophilic polymer surfaces.

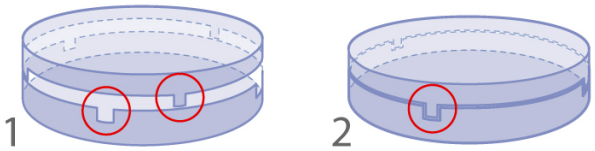
Coating

Specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications or reference.
2. Apply 300 µl and leave at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

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

Using The Lid



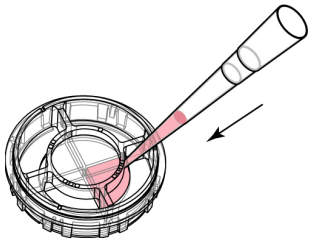
1. open position, easy opening
2. close position, for long term studies, minimal evaporation

Seeding cells

Tip:

The day before seeding the cells we recommend placing the cell medium and the µ–Dish 35mm Quad into the incubator for equilibration. This will prevent the liquid inside wells from emerging air bubbles over the incubation time.

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 1.3–2.7 × 10^4 cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 300 µl cell suspension into each well of the µ–Dish 35mm Quad. If the pipet tip does not reach the depth of each well as shown in the picture, this may result in air bubbles to form and remain under the center plate on the wells. To avoid forming air bubbles, use a gel loader pipet tip whose thin and soft end can reach the depth of the well.



- Avoid shaking as this will result in inhomogeneous distribution of the cells. After cell attachment add additionally 300 µl 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.
- 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 600 µl 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.

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).

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).

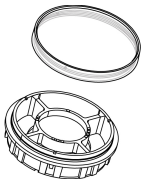
Immersion Oil

When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non–recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are not listed in the table below should be considered as non–compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil	50101	16-12-27	01/2017
Zeiss	Immersol 518 F	444960	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012
Leica	Immersion Liquid	11513859	n.a.	03/2011
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017

Ordering Information

µ–Dish 35mm Quad



Cat. No.	Description
80416	**µ–Dish 35mm Quad ibiTreat**: ⌀ 35 mm, #1.5 polymer coverslip, tissue culture treated, sterilized
80411	**µ–Dish 35mm Quad Uncoated**: ⌀ 35 mm, #1.5 polymer coverslip, 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.