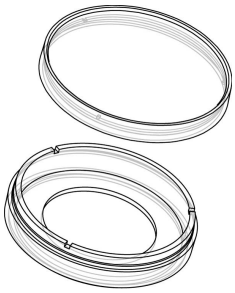


The ibidi product family comprises a variety of different shapes of µ–Slides and µ–Dishes which all have been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments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

The µ–Dish $^{35mm,\ low}$ allows you to perform high resolution microscopy in a 35 mm Petri–dish with 7 mm walls. The low height makes high numerical apertures of Köhler illumination possible and provides large access for micromanipulation. The lid can be closed to hinder evaporation during long term experiments.

Material

ibidi µ–Slides and µ–Dishes consist of a plastic with highest optical quality. The material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the upper part. The µ–Slides and µ–Dishes are not autoclavable since they are temperature stable up to 60°C/140°F only. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the plastic bottom 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

Geometry

Geometry of the µ–Dish $^{35mm,\ low}$	
Diameter dish	35 mm
Volume	800 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	9 mm / 7 mm
Bottom matches coverslip	No. 1.5

Surface and coating

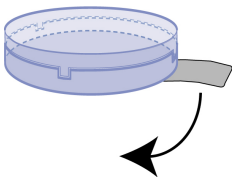
The µ–Dish is available with ibiTreat and uncoated surface. The ibiTreat surface is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested for good cell growth. Uncoated is a very hydrophobic surface and allows no direct cell growth. It is suitable for specific coatings or suspension cells. If, for any reason, you might need special coatings such as Collagen IV, Fibronectin, Poly–L–Lysine, and Poly–D–Lysine these can be provided on request. In this case only high quality substrates are used[1].

The uncoated µ–Dish must be coated to promote cell adhesion. 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 we have observed that 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.

Protection film

Remove the protection film before usage!



The bottom of the µ–Dish is covered with a film to protect the optical quality of the plastic surface. Please pull off the protection film before usage!

[1]Collagen IV, BD Cat.-Nr. 356233, Fibronectin, BD Cat.Nr. 354008, Ploy–L–Lysin, Sigma Cat.-Nr. P4832, Poly–D–Lysin, BD Cat.-Nr. 354219

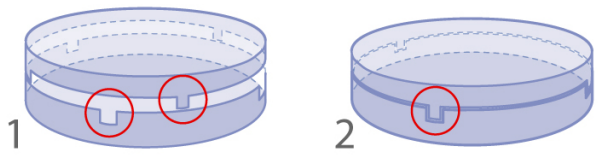
The protection film of all µ–Dishes is color-coded:

Color	Surface	Variation
Blue	ibiTreat	plain
Red	ibiTreat	with Grid–500
Yellow	ibiTreat	with Culture-Insert
White	uncoated	plain
Black	uncoated	with Grid–500
Green	uncoated	with Culture–Insert

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.

Using the lid



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

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 of only 180 µm, 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.

Seeding cells

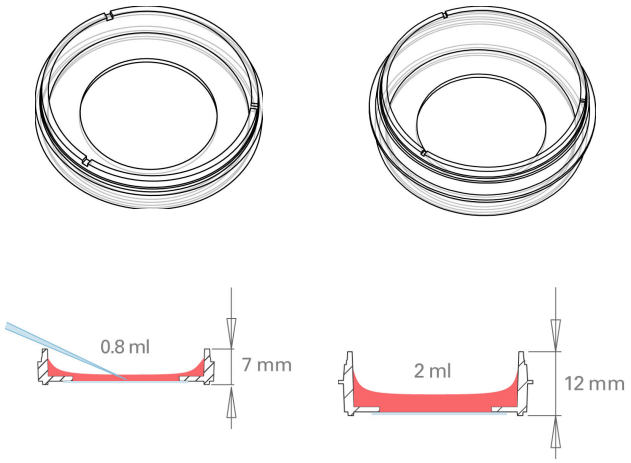
- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 4–9 × 10^4 cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 400 µl cell suspension into the inner well of the µ–Dish. Avoid shaking as this will result in inhomogeneous distribution of the cells. After cell attachment add additionally 400 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.

We recommend not to fill more than 800 µl 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 800 µl fresh medium.

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.



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, Formula Code: 1261	(Cargille) 16242
Zeiss	518 F	(Zeiss) 444960
Olympus	50CC	(Olympus) 35506
Nikon	50 CCM DF	(Nikon) MXA 20351
Leica	immersion oil, low fluorescence	(Leica) 11513859

Selected cell tests on different surfaces

Many eukaryotic and bacterial cells have been tested by ibidi on the different surfaces of the µ-Slides. A variety of other cell lines like COS, CHO, HepG2, and NIH 3T3 were successfully grown by our customers.

	ibiTreat	Collagen IV	Fibronectin	Poly-L-Lysin	Poly-D-Lysin	uncoated
HUVEC	excellent	good	excellent	no cell growth	not done	no cell growth
Rat1	excellent	excellent	excellent	excellent	excellent	poor
HT1080	excellent	excellent	excellent	excellent	not done	poor
HeLa	excellent	excellent	excellent	excellent	not done	poor
Neuro2A	excellent	excellent	excellent	excellent	excellent	poor
PC12	good	excellent	excellent	excellent	excellent	no cell growth
Dictyostelium discoideum	not done	excellent	not done	not done	not done	excellent
Escherichia coli	excellent	not done	not done	excellent	not done	excellent

HUVEC = Human Umbilical Vein Endothelial Cells
Rat1 = Rat Fibroblast
HT1080 = Human Fibrosarcoma
HeLa = Human Cervix Adenocarcinoma
Neuro2A = Mouse Neuroblastoma
PC12 = Rat Pheochromocytom

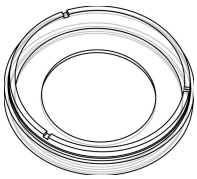
Dictyostelium discoideum = strain wild type AX-2

Escherichia coli = strain MDG131

µ–Dish *35mm, low* family

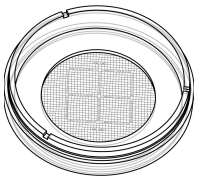
The µ-Dish is available with the ibiTreat-surface and uncoated. Anyhow, please do not hesitate to contact us for other surfaces.

µ–Dish *35mm, low*



Ordering number	Treatment or Coating	Characteristics
80136	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80131	uncoated, sterile	hydrophobic

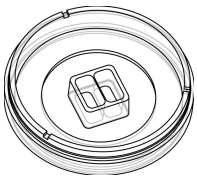
µ–Dish *35mm, low* with Grid-500 *



Ordering number	Treatment or Coating	Characteristics
80156	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80151	uncoated, sterile	hydrophobic

* Please also refer to the Grid-500 instructions.

µ–Dish *35mm, low* with Culture-Insert **



Ordering number	Treatment or Coating	Characteristics
80206	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80201	uncoated, sterile	hydrophobic

** Please also refer to the Culture-Insert instructions.

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.