## Instructions





The ibidi product family is comprised of a variety of  $\mu$ -Slides and  $\mu$ -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  $\mu$ -Dish <sup>35mm, high</sup> 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

ibidi  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -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  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -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 <sub>D</sub> (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.

#### Geometry

Geometry of the µ-Dish <sup>35mm, high</sup>		
Diameter dish	35 mm	
Volume	2000 µl	
Growth area	$3.5 \text{ cm}^2$	
Diameter growth area	21 mm	
Coating area using 400 µl	$4.2 \text{ cm}^2$	
Height with / without lid	14 mm / 12 mm	
Bottom matches coverslip	No. 1.5	

## Shipping and Storage

The  $\mu$ -Slides,  $\mu$ -Dishes and  $\mu$ -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.

Conditi	ons
Shipping conditions	Ambient
Storage conditions	RT (15-25°C)
Shelf Life of Diffe	erent Surfaces
ibiTreat, Glass Bottom, ESS	36 months
Collagen, Poly-L-Lysine	18 months

#### **Surface and Coating**

The  $\mu$ -Dish <sup>35mm, high</sup> 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  $\mu$ -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<sup>2</sup> 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.



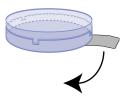
• Aspirate the solution and wash. Optionally, let dry at room temperature.

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

The  $\mu$ -Dish <sup>35mm, high</sup> is also available with an elastically supported surface (ESS) and a glass bottom surface. Please refer to the instructions for detailed information.

## **Protection Film**

#### Remove the protection film before usage!

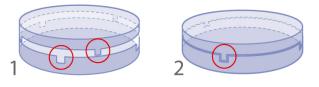


The bottom of the  $\mu$ -Dish is covered with a film to protect the optical quality of the plastic surface. Please pull off the protection film before usage!

#### 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

#### 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  $4-9 \times 10^4$  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  $\mu$ -Dish with the supplied lid. Incubate at 37°C and 5 % CO<sub>2</sub> as usual.

# We recommend not to fill more than the indicated total volume into the $\mu$ -Dish <sup>35mm, high</sup> 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  $\mu$ -Dishes to save space in your incubator. This will not affect cell growth. We recommend making batches with up to 6  $\mu$ -Dishes, due to stability reasons. Placing the  $\mu$ -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  $\mu$ -Dish preferably on an inverted microscope. You can use any fixative of your choice. The  $\mu$ -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  $\mu$ -Dishes and  $\mu$ -Slides (ibidi Mounting Medium, 50001).



## µ–Dish <sup>35mm</sup> Selection Guide

µ–Dish <sup>35mm, low</sup>	µ–Dish <sup>35mm, high</sup>
Low walls (7 mm) for large access to the cells. Designed for micromanipulation and mi- croinjection.	High walls (12 mm) for all stan- dard applications. Also available with glass bot- tom, relocation grid, and elastic surface (ESS).
0.8 ml 7 mm	2 ml 12 mm

## **Minimizing Evaporation**

Using the  $\mu$ –Dish with a closed lid, the evaporation in an incubator system with 37°C and 95% humidity is around 1% per day. Using the  $\mu$ –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 nonrecommended 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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#### 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. © ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.

# Instructions

## **Ordering Information**

### µ–Dish <sup>35mm, high</sup>

	Cat. No.	Description
	81156	$\mu$ -Dish <sup>35mm, high</sup> ibiTreat: $\emptyset$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized
_	81151	<b>µ–Dish</b> <sup>35mm, high</sup> <b>Uncoated</b> : $\emptyset$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, hydrophobic, sterilized

#### µ–Dish <sup>35mm, high</sup> Grid–500

Cat. No.	Description
81166	$\mu$ –Dish <sup>35mm, high</sup> Grid-500 ibiTreat : Ø 35 mm, high wall (2 ml volume), grid repeat distance 500 $\mu$ m, #1.5 polymer coverslip, tissue culture treated, sterilized
81161	$\mu$ –Dish <sup>35mm, high</sup> Grid-500 Uncoated: Ø 35mm, high wall (2 ml volume), grid repeat distance 500 $\mu$ m,#1.5 polymer coverslip, hydrophobic, sterilized

#### Culture–Insert in µ–Dish <sup>35mm, high</sup>

Cat. No.	Description
81176 80366 80466	<b>Culture–Insert 2 Well in µ–Dish</b> <sup>35mm, high</sup> <b>ibiTreat</b> : ready to use, tissue culture treated, sterilized <b>Culture–Insert 3 Well in µ–Dish</b> <sup>35mm, high</sup> <b>ibiTreat</b> : ready to use, tissue culture treated, sterilized <b>Culture–Insert 4 Well in µ–Dish</b> <sup>35mm, high</sup> <b>ibiTreat</b> : ready to use, tissue culture treated, sterilized

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

	Cat. No.	Description
	80406	micro–Insert 4 Well in µ–Dish <sup>35mm, high</sup> ibiTreat: ready to use, tissue culture treated, sterilized
	80486	micro–Insert 4 Well FulTrac in µ–Dish <sup>35mm, high</sup> ibiTreat: ready to use, tissue culture treated, sterilized

#### µ–Dish <sup>35mm, high</sup> Glass Bottom

Cat. No.	Description
81158	$\mu$ –Dish <sup>35mm, high</sup> Glass Bottom: Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 $\mu$ m) D 263 M Schott glass, sterilized

#### µ–Dish <sup>35mm, high</sup> ESS

Cat. No.	Description
81291	<b>μ–Dish <sup>35mm, high ESS 1.5 kPa Uncoated</sup></b> : Ø 35 mm, high wall (2 ml volume), elastic surface with a stiff- ness of 1.5 kPa, hydrophobic, sterilized
81391	$\mu$ –Dish <sup>35mm, high</sup> ESS 15 kPa Uncoated: Ø 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 15 kPa, hydrophobic, sterilized
81191	$\mu$ –Dish <sup>35mm, high</sup> ESS 28 kPa Uncoated: $\emptyset$ 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 28 kPa, hydrophobic, sterilized