

## u-Dish 35 mm, high Grid-500

## 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  $^{35\,\text{mm, high}}$  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.

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 Polymer Coverslip Bottom. The outer dimensions are identical to ibidi μ-Dishes.

#### **Material**

ibidi  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates are made of a polymer 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 intended for one-time use and are not autoclavable, since they are only temperature-stable up to  $80^{\circ}$ C/175°F. Please note that gas exchange between the medium and the 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 4.

## Geometry

| Geometry of the µ-Dish 35 mm, high |                         |
|------------------------------------|-------------------------|
| Diameter dish                      | 35 mm                   |
| Volume                             | 2000 μl                 |
| Growth area                        | $3.5 \text{ cm}^2$      |
| Diameter growth area               | 21 mm                   |
| Coating area using 400 µl          | $4.1 \text{ cm}^2$      |
| Height with / without lid          | 14 mm / 12 mm           |
| Bottom                             | ibidi Polymer Coverslip |

## **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.

| Conditions          |              |  |
|---------------------|--------------|--|
| Shipping conditions | Ambient      |  |
| Storage conditions  | RT (15–25°C) |  |
|                     |              |  |
|                     | Shelf Life   |  |
| ibiTreat, Uncoated  | 36 months    |  |
|                     |              |  |

## **Geometry of the Grid-500**

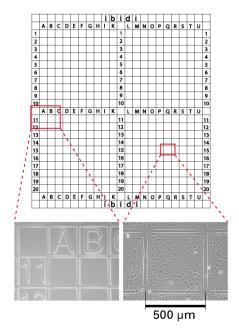
| Geometry of the Grid-500                          |                            |  |
|---|----------------------------|--|
| Number of squares                                 | 400                        |  |
| Repeat distance                                   | $500  \mu m  (\pm  1  \%)$ |  |
| Groove width $40 \mu\text{m} (\pm 5 \mu\text{m})$ |                            |  |
| Groove depth $< 5 \mu m$                          |                            |  |

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

- A to K (I 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



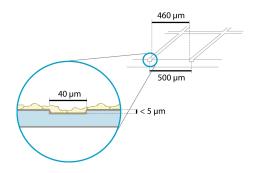
#### 4 x 10 x 10 squares



#### **Characteristics of the Grid**

The Grid-500 is made of small grooves inside the ibidi Polymer Coverslip surface of ibidi  $\mu$ -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  $40 \,\mu\text{m} \ (\pm 5 \,\mu\text{m})$  wide and  $< 5 \,\mu\text{m}$  deep. Cells can grow in the grooves as well. We recommend using objective lenses up to  $20 \times$ . Anyhow, the optical quality meets the requirements of  $63 \times$  and  $100 \times$  oil objective lenses as well (ibidi Polymer Coverslip, No. 1.5).



#### 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

Detailed information about coatings is provided in Application Note 08: Coating protocols for ibidi labware products.

In short, specific coatings are possible following this protocol:

- 1. Prepare your coating solution according to the manufacturer's specifications or reference.
- 2. Apply  $400\,\mu l$  and leave at room temperature for at least 30 minutes.
- 3. Aspirate the solution and wash with the recommended protein dilution buffer.
- 4. The μ-Dish <sup>35 mm, high</sup> Grid-500 is ready to be used. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

#### 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.

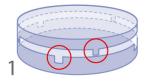
- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration.
- Apply 400 µl cell suspension into the inner well of the µ-Dish. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- After cell attachment add additionally 1.6 ml of pure medium to ensure optimal grow conditions.
- Cover the  $\mu\text{-Dish}$  with the supplied lid. Incubate at 37°C and 5 % CO2 as usual.

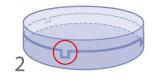


We do not recommend filling more than 2 ml into the  $\mu$ -Dish  $^{35\,\text{mm},\,\text{high}}$  Grid-500 in order to avoid the liquid contacting the lid.

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 fresh medium.

## **Using The Lid**





- 1. Open position, easy opening
- 2. Close position, for long term studies, minimal evaporation

## **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).

## 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.

## **Microscopy**

To analyze your cells, no special preparations are necessary. Cells can be directly observed live or fixed, prefer-

ably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and storage of fixed and stained samples, ibidi provides a mounting medium (50001) optimized for  $\mu$ -Dishes,  $\mu$ -Slides, and  $\mu$ -Plates.

## **Chemical Compatibility**

The following table provides some basic information on the chemical and solvent compatibility of the  $\mu$ -Dish  $^{35\,\text{mm},\,\text{high}}$  Grid-500. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on ibidi.com.

| Chemical / Solvent | Compatibility                       |
|--------------------|-------------------------------------|
| Methanol           | yes                                 |
| Ethanol            | yes                                 |
| Formaldehyde       | yes                                 |
| Acetone            | no                                  |
| Mineral oil        | no                                  |
| Silicone oil       | yes                                 |
| Immersion oil      | See <b>Immersion Oil</b> on page 4. |

## μ-Dish 35 mm Selection Guide

| μ-Dish <sup>35 mm, low</sup>   | μ-Dish 35 mm, high                                |
|--|---|
| Low walls (7 mm) for large access to the cells. Designed for micromanipulation and microinjection. | High walls (12 mm) for all standard applications. |
|  |   |
| 0.8 ml 7 mm  | 2 ml 12 mm  |



# $\mu$ -Dish $^{35\,mm,\,high}$ Grid-500

# Instructions

## **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   |
| Cargille  | Type A                      | 16482         | 100592     | 01/2017   |
| Cargille  | Type HF                     | 16245         | 92192      | 01/2017   |
| Carl Roth | Immersion oil               | X899.1        | 414220338  | 01/2017   |
| Leica     | Immersion Liquid            | 11513859      | n.a.       | 03/2011   |
| Nikon     | Immersion Oil F2 30cc       | MXA22192      | n.a.       | 01/2020   |
| Nikon     | Silicone Immersion Oil 30cc | MXA22179      | 20191101   | 01/2020   |
| Olympus   | Silicone Immersion Oil      | SIL300CS-30CC | N4190800   | 01/2017   |
| Zeiss     | Immersol 518 F              | 444960        | 160706     | 01/2017   |
| Zeiss     | Immersol W 2010             | 444969        | 101122     | 04/2012   |



# Instructions

# $\mu\text{-Dish}~^{35\,mm,\,high}$ Grid-500

# **Ordering Information**

# μ-Dish <sup>35 mm, high</sup>



| Cat. | No.   | Description  |
|------|-------|--|
| 8115 | 6     | $\mu$ -Dish $^{35 \text{ mm, high}}$ ibiTreat: $\varnothing$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized          |
| 8115 | 6-400 | $\mu$ -Dish $^{35 \text{ mm, high}}$ ibiTreat, Bulk Pack: $\emptyset$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, tissue culture treated, sterilized |
| 8115 | 1     | $\mu$ -Dish $^{35\text{mm, high}}$ Uncoated: $\varnothing$ 35 mm, high wall (2 ml volume), #1.5 polymer coverslip, hydrophobic, sterilized                       |

#### μ-Dish 35 mm, high Grid-500



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

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



| Cat. No.  | Description  |
|-----------|--|
| 81158     | $\mu$ -Dish <sup>35 mm, high</sup> Glass Bottom: Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized            |
| 81158-400 | $\mu$ -Dish <sup>35 mm, high</sup> Glass Bottom, Bulk Pack: Ø 35 mm, high wall (2 ml volume), #1.5H (170 ±5 μm) D 263 M Schott glass, sterilized |

## $\mu\text{-Dish}$ $^{35\,mm,\,high}$ ESS



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

# For research use only!

Further information 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.

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