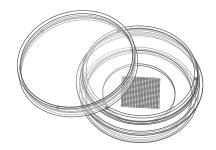


μ-Dish 35 mm, high Grid-500

Instruction Manual



The ibidi labware is comprised of a variety of μ -Slides, μ -Dishes, and μ -Plates, which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the ibidi Polymer Coverslip is similar to that of glass, enabling a variety of microscopy techniques with uncompromised resolution and choice of wavelength.

The μ -Dish $^{35\,\text{mm},\,\tilde{h}igh}$ Grid-500 features a grid structure designed for precise localization, counting, and tracking of individual cells in a defined area. It offers 400 distinct observation squares, each with an edge length of 500 μ m. The grid is easily visible in phase contrast microscopy.

This document applies to the following product:

81166 µ-Dish 35 mm, high Grid-500 ibiTreat

Material

The μ -Dish ^{35 mm, high} Grid-500 is made of a polymer that has the highest optical quality. The ibidi Polymer Coverslip bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. It is not possible to detach the bottom from the upper part. The dish is intended for one-time use and is not autoclavable, since it is only temperature-stable up to 80 °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 of Polymer Coverslip Refractive index (589 nm) 1.52

Abbe number	56
Thickness	No. 1.5 (180 μm)
Material	Polymer



WARNING – The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found in the Section "Immersion Oil".

Shipping and Storage

This product is sterilized and sealed in a gaspermeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

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

	Shelf Life
ibiTreat	36 months

Geometry

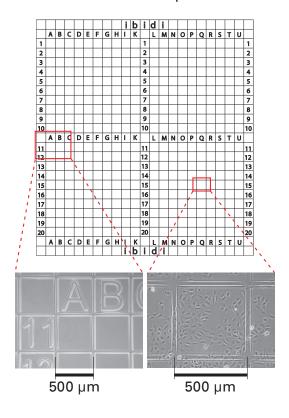
Specifications of the μ-Dish ^{35 mm, high}				
ø μ-Dish	35 mm			
Volume	2 ml			
Growth area	$3.5\mathrm{cm}^2$			
ø observation area	21 mm			
Coating area	4.1 cm ²			
Height with / without lid	14 mm / 12 mm			
Bottom	ibidi Polymer Coverslip			

Specifications of the Grid-500			
Number of squares	400		
Repeat distance	500 μm (± 1%)		
Groove width	40 μm (± 5 μm)		
Groove depth	$<$ 5 μm		

The four major squares are divided into 10×10 observation fields, labeled with letters and numbers ranging from:

- A to K (excluding J) and 1 to 10
- A to K (excluding J) and 11 to 20
- L to U and 1 to 10
- L to U and 11 to 20

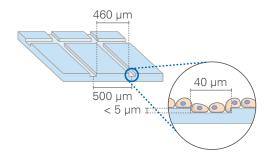
$4 \times 10 \times 10$ squares



Microscopic images of the grid. Left: brightfield microscopy without cells, $4\times$ objective lens. Right: phase contrast microscopy with rat fibroblasts, $10\times$ objective lens.

Characteristics of the Grid-500

The Grid-500 incorporates small grooves on the ibidi Polymer Coverslip surface. These grooves are imprinted on the side where cells are cultured. They do not affect any cell growth, coating protocols, or the surface's properties. Cell proliferation and behavior remain comparable to that observed in standard non-gridded dishes. Both cells and the grid are positioned in the same focal plane. The grid's grooves are approximately 40 μm wide ($\pm\,5\,\mu m$) and less than $5\,\mu m$ deep, allowing cells to also grow within the grooves. While lenses up to $20\times$ are recommended, the optical quality is sufficient for $63\times$ and $100\times$ oil objective lenses as well.



Surface

The μ -Dish $^{35\,\text{mm},\,\text{high}}$ Grid-500 is available with either an ibiTreat or an Uncoated surface.

The tissue culture-treated, hydrophilic ibiTreat surface of the ibidi Polymer Coverslip is ideal for culturing adherent cells. It ensures excellent cell adhesion without the necessity for any additional coatings. Nonetheless, extracellular matrix (ECM) protein coatings can be applied to the ibiTreat surface without any restrictions, if required.

The hydrophobic Uncoated surface of the ibidi Polymer Coverslip offers weak cell adhesion unless pre-coated with an ECM protein. You can apply coatings to the Uncoated surface without any restrictions. This surface is suitable for culturing adherent cells that require a specific coating.

For establishing a particular coating, we advise testing your procedure on both ibiTreat and Uncoated surfaces, as proteins and biomolecules may adhere differently to hydrophilic or hydrophobic surfaces.

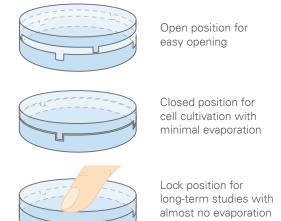
Coating

Detailed information about coatings is provided in Application Note 08: Coating Protocols for ibidi Labware.

In short, specific coatings are possible following this protocol:

- Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 4.1 cm² and a volume of 400 µl.
- Apply 400 µl into the central growth area. Make sure that the entire bottom of the dish is covered with liquid by gently tilting or shaking it. Close the lid and leave the dish at room temperature for at least 30 minutes.
- 3. Aspirate the solution and wash with the recommended protein dilution buffer.
- 4. The coated dish is ready to be used. Be aware that allowing the dish to dry out is not recommended, as some coating proteins may degrade upon drying.

Lid with Locking Feature for Minimized Evaporation



Seeding Cells

- Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 4–9 × 10⁴ cells/ml suspension should result in a confluent layer within 2–3 days.
- 2. Apply 400 µl cell suspension into the growth area of the dish. Avoid shaking, as this will result in inhomogeneous cell distribution.
- After cell attachment, add 1.6 ml of medium to ensure optimal growing conditions.
- 4. Cover the dish with the supplied lid. Incubate as usual (e.g., at 37 ℃ and 5% CO₂).



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

Insensitive cells can be left in their seeding medium for several days and grow to confluence there. However, optimal results might be achieved when the medium is changed every 2–3 days. For this, carefully aspirate the old medium and replace it by up to 2 ml fresh medium.



TIP – You can stack the μ -Dishes to save space in your incubator. This will not affect cell growth. Due to stability reasons, we recommend making batches with not more than 6 μ -Dishes. Placing the μ -Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination each time the incubator is opened.

Chemical Compatibility

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

Chemical / Solvent	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	No
Mineral oil	No
Silicone oil	Yes
Immersion oil	See Section "Immersion Oil"

Microscopy

To image your cells, no special preparations are necessary. Living or fixed cells can be directly observed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and for storage of fixed and stained samples, ibidi provides mounting media that are optimized for ibidi labware:

Cat. No. 50001: ibidi Mounting Medium

Cat. No. 50011: ibidi Mounting Medium with DAPI

Immersion Oil



WARNING – 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 2	50102	24-07-04	07/2024
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.	03/2023
Leica	Immersion Liquid Type G	11513910	n.a.	04/2024
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	444960-0000	220211	03/2023
Zeiss	Immersol 518 F (30 °C)	444970-9010	220816	03/2023
Zeiss	Immersol 518 F (37°C)	444970-9000	220302	03/2023
Zeiss	Immersol W 2010	444969-0000	101122	04/2012
Zeiss	Immersol Sil 406	444971-9000	80730	03/2023
Zeiss	Immersol G	462959-9901	211117	03/2023

For research use only!

Further information can be found at ibidi.com. For questions and suggestions, please contact us by e-mail at info@ibidi.com or by telephone at +49 (0)89/520 4617 0.

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