

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 glass bottom versions are especially designed for TIRF, super-resolution, and single molecule applications. The μ-Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom features a grid structure designed for precise localization, counting, and tracking of individual cells in a defined area. It offers 4 × 400 distinct observation squares, each with an edge length of 50 μm. The grid is easily visible in phase contrast microscopy.

This document applies to the following product:

81148 **μ-Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom**

## Material

The μ-Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom is made with a glass coverslip bottom. 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.

### Optical Properties of Glass Coverslip

Refractive index	1.523
Abbe number	55
Thickness	No. 1.5H (170 μm ± 5 μm)
Material	Schott borosilicate glass, D 263 M



**CAUTION** – Be cautious when handling ibidi labware products with a glass bottom! The glass coverslip or slide is fragile and can break easily. Handle these items carefully to prevent physical injury and damage to devices due to medium leakage.

## Shipping and Storage

This product is sterilized and sealed in a gas-permeable 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

Glass Bottom	36 months
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## Geometry

### Specifications of the μ-Dish<sup>35 mm, high</sup>

∅ μ-Dish	35 mm
Volume	2 ml
Growth area	3.5 cm <sup>2</sup>
Coating area	4.1 cm <sup>2</sup>
∅ observation area	21 mm
Height with / without lid	14/12 mm
Bottom	Glass coverslip No. 1.5H

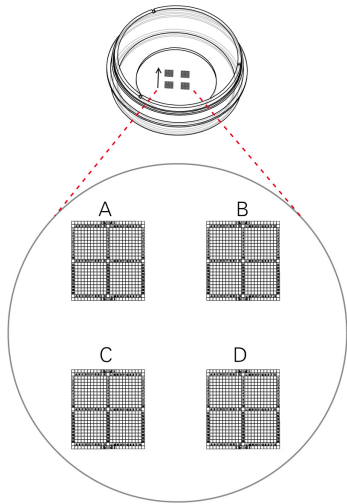
### Specifications of the Grid-50

Number of squares	4 × 400
Repeat distance	50 μm (± 1%)
Groove width	5 μm (± 1 μm)
Groove depth	< 5 μm

There are four grids labeled A to D. Each consists of four major squares which are divided into 10 × 10 observation fields, labeled with letters and numbers ranging from:

- A to K (excluding J) and 1 to 10

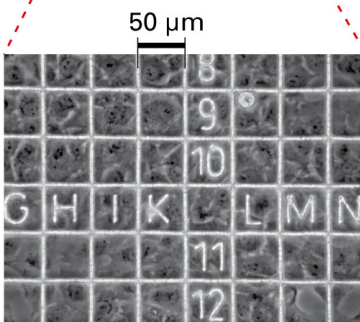
- A to K (excluding J) and 11 to 20
- L to U and 1 to 10
- L to U and 11 to 20



There are four grids labeled A to D.

4 x 10 x 10 squares  
**A**

	i b i d i										L M N O P Q R S T U									
A	B	C	D	E	F	G	H	I	K	L	M	N	O	P	Q	R	S	T	U	
1										1									1	
2										2									2	
3										3									3	
4										4									4	
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11	A	B	C	D	E	F	G	H	I	K	L	M	N	O	P	Q	R	S	T	U
11										11									11	
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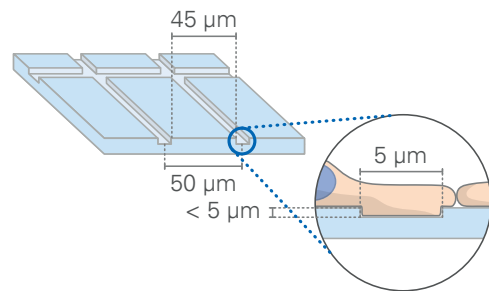


Microscopic image of the grid with rat fibroblasts, phase contrast, 20x objective lens.

## Characteristics of the Grid-50

The Grid-50 incorporates small grooves on the glass 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 5 μm wide (± 1 μm) and less than 5 μm deep, allowing cells to also grow within the grooves. While lenses up to 20x are recommended, the optical quality is sufficient for 63x and 100x oil objective lenses as well.



## Surface

The μ-Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom is manufactured with a glass coverslip. Washing it (e.g., with PBS) before cell seeding helps removing glass dusts, which enhances direct cell growth on the surface.

## Coating

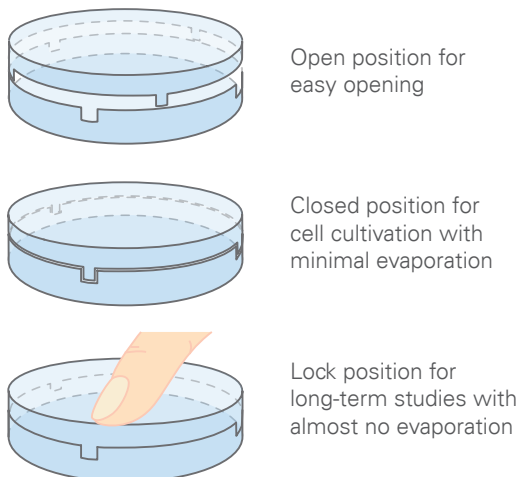
Detailed information about coatings is provided in [Application Note 08: Coating Protocols for ibidi Labware](#).

In short, specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 4.1 cm<sup>2</sup> and a volume of 400 μl.

2. Apply 400  $\mu$ l 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

1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a  $4-9 \times 10^4$  cells/ml suspension should result in a confluent layer within 2–3 days.
2. Apply 400  $\mu$ l cell suspension into the growth area of the dish. Avoid shaking, as this will result in inhomogeneous cell distribution.
3. 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°C and 5% CO<sub>2</sub>).



**CAUTION** – We do not recommend filling more than 2 ml into the  $\mu$ -Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom 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  $\mu$ -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  $\mu$ -Dishes. Placing the  $\mu$ -Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination each time the incubator is opened.

### Optional: Glass Coverslip Cleaning

The  $\mu$ -Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom is made with an uncoated glass coverslip. For special applications, the coverslip of the dish can be cleaned by following the protocol below.

1. Remove lids and immerse the dish in ddH<sub>2</sub>O in an appropriately sized beaker.
2. Sonicate for 10 minutes.
3. Decant the ddH<sub>2</sub>O completely.
4. Add 1 M HCl and sonicate for 10 minutes.
5. Decant the HCl completely and wash twice with ddH<sub>2</sub>O. Decant the ddH<sub>2</sub>O completely.
6. Add absolute 2-propanol and sonicate for 10 minutes.

7. Aspirate the 2-propanol completely. Make sure that all products are completely dry. Wash twice with ddH<sub>2</sub>O and aspirate the ddH<sub>2</sub>O completely.
8. Add absolute ethanol and sonicate for 10 minutes.
9. Aspirate the ethanol completely. Make sure that all products are completely dry. Wash twice with ddH<sub>2</sub>O.
10. Sonicate in ddH<sub>2</sub>O for 10 min.
11. Decant ddH<sub>2</sub>O and blow dry carefully with canned air or clean nitrogen gas.

Modifications of this protocol including acids, bases, alcohols, and detergents are possible, please check the Section “Chemical Compatibility”. Make sure to handle the glass-bottomed products with care. The glass coverslips may break during mechanical handling. For optimal results, use a custom-made teflon holder.

### Immersion Oil

When using ibidi Glass Bottom products with oil immersion objectives, there is no known incompatibility with any immersion oil on the market. All types of immersion oils can be used.

### 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](#)

### Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the μ-Dish<sup>35 mm, high</sup> Grid-50 Glass Bottom. For a full list of compatible solvents and more information on chemical compatibility, visit [ibidi.com/chemicals](http://ibidi.com/chemicals).

Chemical / Solvent	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	Yes, without lid
Mineral oil	Yes
Silicone oil	Yes
Immersion oil	See Section “Immersion Oil”

### For research use only!

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

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