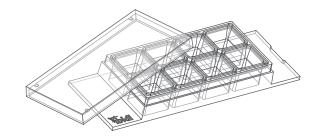
μ-Slide 8 Well high μ-Pattern ibiTreat, cir500, pit1000, hex

cells in focus bidi®

Instruction Manual



The ibidi labware is comprised of a variety of $\mu\textsc{-Slides}$, $\mu\textsc{-Dishes}$, and $\mu\textsc{-Plates}$, 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 -Patterning technology enables spatially defined cell adhesion for various 2D and 3D cell culture applications. The cell-adhesive patterns are surrounded by the non-adhesive Bioinert surface on the ibidi Polymer Coverslip, allowing for precisely controlled cell adhesion. The μ -Patterns are dry-stable, sterile, and ready to use.

This document applies to the following product:

83813 μ-Slide 8 Well high μ-Pattern ibiTreat, cir500, pit1000, hex

Material

The μ -Slide 8 Well high μ -Pattern 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 slide 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 CoverslipRefractive index (589 nm)1.52Abbe number56ThicknessNo. 1.5 (180 μm)MaterialPolymer



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.

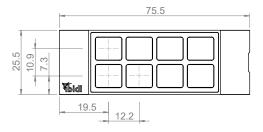
Conditions			
Shipping conditions	Ambient		
Storage conditions	RT (15–25 ℃), dry place (relative humidity <50%)		

Shelf Life		
μ-Patterning	36 months	

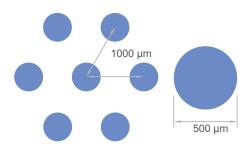
Geometry

This product provides a standard slide format according to ISO 8037/1.

Specifications of the μ-Slide 8 Well high				
Outer dimensions (w x I)	$25.5 \times 75.5 \text{mm}^2$			
Number of wells	8			
Dimensions of wells	$9.4 \times 10.7 \times 9.3$			
$(w \times l \times h)$	mm ³			
Volume per well	300 μΙ			
Height with/without lid	10.8 / 9.5 mm			
Growth area per well	0.22 cm ²			
Coating area per well	0.22 cm ²			
Bottom	ibidi Polymer Coverslip			



Specifications of the μ-Pattern				
Binding motif	ibiTreat (tissue			
	culture-treated)			
Pattern shape	Circle			
Diameter	500 μm			
Pitch	1000 μm			
Pattern layout	Hexagonal			
Number of patterns	ca. 115 / well			

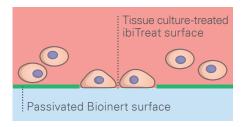




NOTE – Due to the Bioinert passivation, only the patterns can be coated with proteins or seeded with cells. Therefore, growth area and coating area are identical.

Surface

The cell-adhesive patterns are surrounded by the non-adhesive Bioinert surface on the ibidi Polymer Coverslip. The patterns are slightly visible under the phase contrast microscope.



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 protein coatings can be applied to the ibiTreat surface without any restrictions, if required.

The Bioinert surface is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip. It allows no adsorption, coating, or binding of proteins, antibodies, enzymes, and other biomolecules. Therefore, the Bioinert technology provides a stable passivation in cell-based assays for several days or even weeks. The hydrophilic Bioinert surface hinders any protein attachment, thus inhibiting subsequent cell attachment. The Bioinert surface is not biodegradable by cells allowing long-term assays with suspension cells and cell aggregates, such as spheroids, organoids, and embryoid bodies.

Characteristics of the Bioinert Surface				
Bioinert surface thickness	200 nm			
Bioinert surface material	Polyol-based			
	hydrogel			
Protein coatings	Not possible			

Coating



NOTE – The coating step is optional.



NOTE – The coating concentration described here is a nominal starting point. Depending on the proteincell combination, adjustments may be needed to optimize cell attachment: increase the concentration if cells do not adhere properly to the pattern; decrease it if cells attach outside the patterned area.

The coating area of the μ -Slide 8 Well high μ -Pattern ibiTreat, cir500, pit1000, hex is 10 times smaller than that of the non-patterned μ -Slide. Consequently, the recommended coating concentration is also 10 times lower. To determine the nominal concentration, divide the value from Application Note 08: Coating Protocols for ibidi Labware by 10. After that, follow this protocol:

- Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 0.22 cm² and a volume of 300 µl per well.
- 2. Apply 300 µl per well and leave it at room temperature for at least 1 hour; optionally at 37 ℃.
- Aspirate the solution and wash three times thoroughly with the recommended protein dilution buffer to remove any unbound proteins.
- 4. The coated slide is ready to be used. Be aware that allowing the coated surface to dry out is not recommended.



NOTE – To dilute collagen coating solutions, use 50 mM (\sim 0.18%) HCl instead of acetic acid. While this may differ from some manufacturers' protocols and the ibidi Application Note 08, HCl has shown improved coating performance on the ibiTreat μ -Pattern.

Seeding Cells

- Prepare your cell suspension or spheroids as usual. Depending on your needs, we recommend a $2-14 \times 10^4$ cells/ml single cell suspension or a spheroid suspension with $0.75-4.5 \times 10^3$ spheroids/ml.
- Apply 300 μl cell suspension per well.
 Avoid shaking, as this will result in inhomogeneous cell distribution.
- Cover the slide with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO₂).
- After cell attachment, wash with cell-free medium to remove non-attached cells and debris.

Insensitive cells can be left in their seeding medium for several days. However, optimal results might be achieved when the medium is changed every 1–2 days. For this, carefully aspirate the old medium and replace it by 300 µl fresh medium per well. For sensitive cells, exchanging only 50% of the old medium is recommended.

For more information about optimization of cell adhesion and pattern coverage, please refer to Application Note 65: Cell Adhesion on ibidi μ-Patterns: Parameters and Optimization.



CAUTION – Ensure incubator shelves and microscope stages are level, as single cells or clusters may gradually shift to one side on uneven surfaces. Please also avoid evaporation and temperature changes. Both will lead to convectional flow.

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 -Slide 8 Well high μ -Pattern. 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	Yes, without lid
Mineral oil	No
Silicone oil	Yes
Immersion oil	See Section "Immersion Oil"

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

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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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