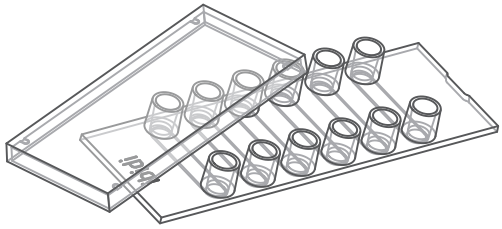
µ-Slide VI 0.4
µ-Pattern ibiTreat, cir500, pit1000, hex
Instruction Manual

cells in focus ibidi®



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-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. The µ-Slide VI 0.4 supports static or flow-based cell culture, immunofluorescence assays, and imaging of fixed or living cells.

This document applies to the following product:

83613	**µ-Slide VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex**

Material

The µ-Slide VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex 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 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 gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

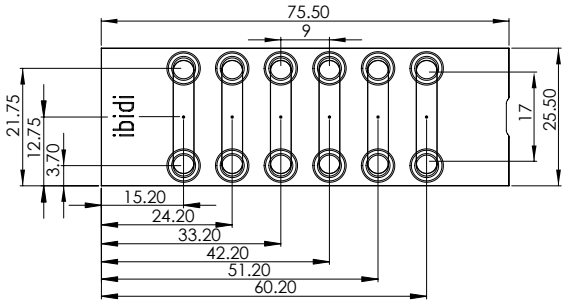
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C), dry place (relative humidity <50%)

Shelf Life	
µ-Patterning	36 months

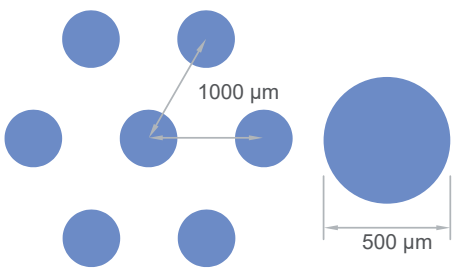
Geometry

The µ-Slide VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex provides a standard slide format according to ISO 8037/1. The 9 mm lateral adapter-to-adapter distance (as in 96 well plates) enables the use of multichannel pipettes.

Specifications of the µ-Slide VI 0.4	
Outer dimensions (w × l)	25.5 × 75.5 mm²
Adapters	Female Luer
Number of channels	6
Channel height	0.4 mm
Channel length	17 mm
Channel width	3.8 mm
Volume of each channel	30 µl
Volume per adapter	60 µl
Height with/without lid	8.7 / 7.5 mm
Growth area per channel	0.15 cm²
Coating area per channel	0.15 cm²
Bottom	ibidi Polymer Coverslip



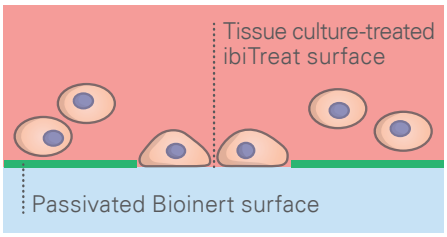
Specifications of the µ-Pattern	
Binding motif	ibiTreat (tissue culture-treated)
Pattern shape	Circle
Diameter	500 µm
Pitch	1000 µm
Pattern layout	Hexagonal
Number of patterns	ca. 75 / channel



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.

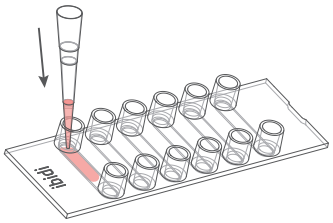
The Bioinert surface is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip. It allows no adsorption, coating, or binding of proteins, antibodies, enzymes, and other biomolecules. Therefore, the Bioinert technology provides a stable passivation in cell-based assays for several 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

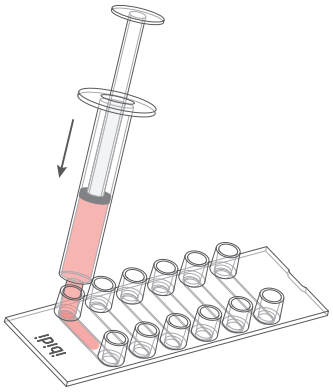
Filling Channel Slides

To avoid air bubbles inside the channel, please follow the recommendations below.

When filling the channel (e.g., with cell suspension or coating solution), place the pipet tip directly at the channel's inlet and dispense the volume with a constant and swift flow.

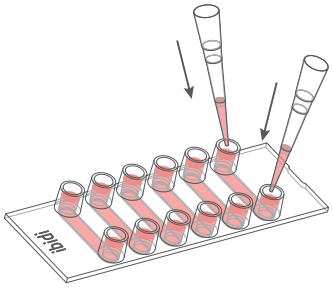


In certain cases, such as when the channel surface is hydrophobic or when filling small channels, it may be necessary to use a syringe. Choose a low-volume syringe with a capacity of 1 or 2.5 ml.



CAUTION – When seeding cells, only add the exact volume needed for the channel. Avoid excess cell suspension in the reservoirs.

After cell attachment, fill 60 µl cell-free medium into each Luer reservoir as shown. Do not trap air bubbles.



Medium Exchange

The following protocol for continuous medium exchange should be applied for cell culture medium replacement, staining, and washing procedures.

1. Remove the medium from the reservoirs with a pipet, putting the pipet tip away from the channel inlet. Do not remove any liquid from the channel itself.
2. Slowly, fill 120 µl fresh medium into one of the reservoirs, which will replace the channel volume by gravity flow. Aspirate from the other reservoir by carefully using a pipet.
3. For a 99% exchange, repeat the steps 1 and 2 three times.
4. Refill the reservoirs using 60 µl per reservoir.

CAUTION – Be careful when using a cell culture aspiration device, as this may flush away partially attached cells or clusters.

CAUTION – Take care that the channel never falls dry during the exchange process. This helps avoiding air bubbles.

TIP – The day before seeding the cells, we recommend placing the cell medium, the slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles during the incubation time.
Quick dispensing of cell suspension helps to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.

Coating

NOTE – The coating step is optional.

NOTE – The coating concentration described here is a nominal starting point. Depending on the protein-cell 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 VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex is 8 times smaller than that of the non-patterned µ-Slide. Consequently, the recommended coating concentration is also 8 times lower. To determine the nominal concentration, divide the value from Application Note 08: Coating Protocols for ibidi Labware by 8. After that, follow this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 0.15 cm^2 and a volume of 30 µl per channel.
2. Apply 30 µl per channel and leave it at room temperature for at least 1 hour; optionally at 37 °C.
3. 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.

TIP – If direct cell seeding is required after coating, seed cells without emptying the channel. For this, follow the protocol in the Section "Medium Exchange" using cell suspension.

NOTE – To dilute collagen coating solutions, use 50 mM (∼ 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

1. Prepare your cell suspension or spheroids as usual. Depending on your cell type, we recommend a 1–7 × 10^5 cells/ml single cell suspension or a suspension of spheroids with 0.4–3.6 × 10^4 spheroids/ml.
2. Add 30 µl cell or spheroid suspension directly into each channel. Quick dispensing helps to avoid trapped air bubbles.
3. Cover the slide with the supplied lid and incubate as usual (e.g., at 37 °C and 5% CO_2).
4. After cell attachment, wash with cell-free medium to remove non-attached cells and debris.
5. Fill each reservoir with 60 µl medium.

We recommend exchanging the medium every day in static culture, following the protocol in the Section "Medium Exchange".

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.

TIP – Trapped air bubbles can be removed from the channel by inclining the slide and knocking at one edge.

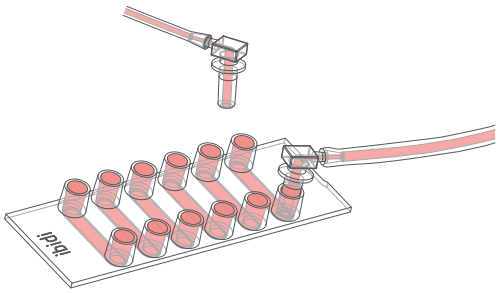
TIP – For longer cultivation, instead of changing medium regularly, you could use a perfusion system or an incubator-compatible cell culture rocker.

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.

Connecting Tubing for Perfusion

The µ-Slide VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex is compatible with the ibidi Pump System and other pump setups for cell cultivation under flow. For this, cells are seeded into the channel and the flow is applied after cell attachment.

1. Fill both Luer ports of the designated flow channel completely with cell-free medium. This ensures air bubble-free connection of the tubing.
2. Prepare the perfusion system: Fill the tubing completely with medium, then pinch it off using a screw clamp or hose clip.
3. Connect the male Luer ends of the clamped tubing to the Luer ports one at a time, ensuring no air is trapped. Remove any excess medium with a tissue.



4. Open the clamped tubing and conduct your perfusion experiment.

For a serial connection of several µ-Slides VI 0.4 with each other, please refer to Application Note 31: Serial Connection of µ-Slide VI 0.4 Channels for Flow Experiments.

Shear Stress Calculations

Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11: Shear Stress and Shear Rates for ibidi µ-Slides.

To calculate the shear stress (τ) in µ-Slide VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex, insert the flow rate (Φ) and the dynamic viscosity (η) in the formula provided below:

$$\tau = \eta \cdot 176.1 \cdot \Phi$$

For simplicity, the calculations include conversions of units (not shown). Please insert the values in the unit definitions given below:

Shear stress	$\tau \left[\frac{dyn}{cm^2}\right]$
Dynamical viscosity	$\eta \left[\frac{dyn \cdot s}{cm^2}\right]$
Flow rate	$\Phi \left[\frac{ml}{min}\right]$

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 VI 0.4 µ-Pattern ibiTreat, cir500, pit1000, hex. 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

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.