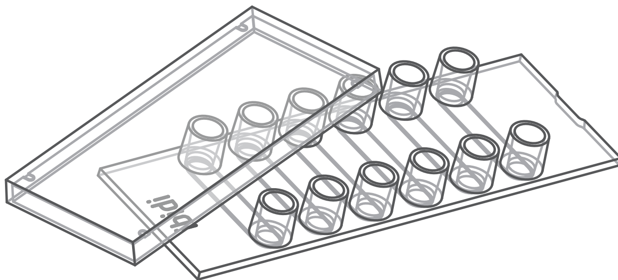
μ-Slide VI 0.4
μ-Pattern ibiTreat, sqr30, pit75, hex
Instruction Manual

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 convenient six channel format of the μ-Slide VI 0.4 is ideal for static cell cultivation and the application of standard immunofluorescence protocols, like treatment, staining, and microscopy of living or fixed cells. Alternatively, the μ-Slide VI 0.4 can be connected to a pump and enables you to observe cells under flow conditions.
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 is applicable to the following product:

83611	**μ-Slide VI 0.4 μ-Pattern ibiTreat, sqr30, pit75, hex**

Material

The μ-Slide VI 0.4 μ-Pattern ibiTreat, sqr30, pit75, 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)

Shelf Life	
μ-Patterning	36 months

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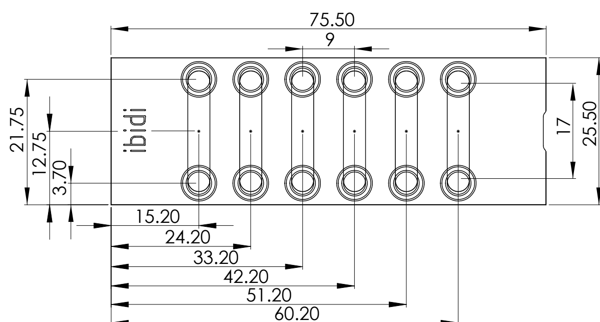
Store the µ-Patterning products in a dry place (relative humidity <50%).

Geometry of the µ-Slide VI 0.4

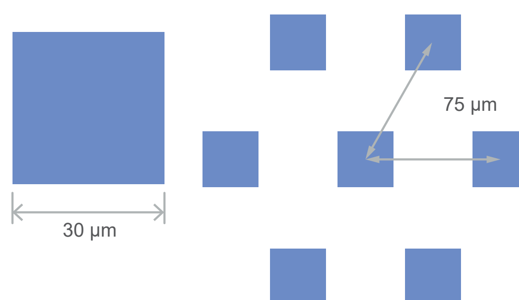
The µ-Slide VI 0.4 provides a standard slide format according to ISO 8037/1. The lateral adapter to adapter distance of 9 mm (like 96 well plates) allows using multichannel pipettes.



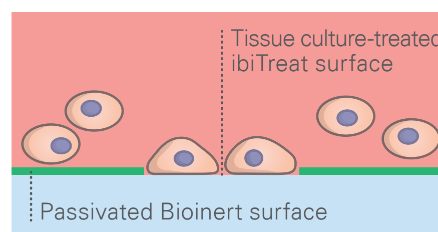
Geometry	
Outer dimensions in mm (w × l)	25.5 × 75.5
Adapters	Female Luer
Number of channels	6
Channel volume	30 µl
Channel height	0.4 mm
Channel length	17 mm
Channel width	3.8 mm
Volume per adapter	60 µl
Height with/without lid	8.7/7.5 mm
Growth area	0.12 cm² per channel
Coating area using 30 µl	0.12 cm² per channel
Bottom	ibidi Polymer Coverslip

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. Please also keep in mind that the coating area is 10 times smaller compared to the non-patterned µ-Slide. Consequently, the recommended coating concentration is 10 times lower as described in Application Note 08: Coating Protocols for ibidi Labware

Specifications of the µ-Pattern	
Binding motif	ibiTreat (TC-treated)
Pattern shape	Square
Side length	30 µm
Pitch	75 µm
Pattern layout	Hexagonal
Number of patterns	ca. 13250 / channel



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.



Surface

The Bioinert surface is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip. In contrast to standard ultra-low attachment coatings, Bioinert is completely non-adherent and allows no binding of any biomolecule, even in long-term experiments. This makes Bioinert ideal for culture and high-resolution imaging of suspension cells and cell aggregates, such as spheroids, organoids and embryoid bodies.

Miniaturized adhesive patterns are irreversibly printed in the Bioinert surface of the ibidi Polymer Coverslip, allowing for precisely controlled cell adhesion. The µ-Patterns are dry-stable, sterile, and ready to use.

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

Coating

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

Please keep in mind that the coating area of the µ-Slide VI $^{0.4}$ µ-Pattern $^{\text{ibiTreat, sqr30, pit75, hex}}$ is 10 times smaller compared to the non-patterned µ-Slide. Consequently, the recommended coating concentration is 10 times lower as described in Application Note 08. Divide the concentration from Application Note 08 by 10 in order to get the recommended nominal coating concentration for the µ-Slide VI $^{0.4}$ µ-Pattern $^{\text{ibiTreat, sqr30, pit75, hex}}$.

After that, follow this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 0.12 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 60 minutes; 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.

NOTE – The coating step is optional.

NOTE – The coating concentration described here is a first recommendation, i.e. a nominal coating concentration. Depending on the protein-cell combination, adjustments might be necessary to optimize cell attachment:
Increase the nominal coating concentration if cells do not attach properly on the patterned surface.
Decrease the nominal coating concentration if there is cell attachment outside of the patterned surface.

NOTE – For diluting collagen coating solutions, please use 50 mM (∼ 0.18%) HCl. Please do not dilute collagen with acetic acid. For some collagens, this is a deviation from the manufacturers protocol and also from our Application Note 08 but the use of HCl showed better coating results on the ibiTreat µ-Pattern.

Seeding Cells

Follow the steps below as a guideline for a general cell application protocol. Please optimize the cell concentration for your needs in subsequent experiments.

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, we recommend a 1–7 × 10^5 cells/ml suspension.
- Apply 30 µl cell suspension into the channel of the µ-Slide. Quick dispensing helps to avoid trapped air bubbles.
- Cover with the supplied lid and incubate at 37 °C and 5 % CO_2 as usual.
- Await cell attachment.
- After 4 hours, carefully fill each reservoir with ca. 60 µl cell-free medium.

- After 4 – 24 hours, wash with cell-free medium to remove non-attached cells and debris. Leave each reservoir filled with 60 µl cell-free medium.

NOTE – The day before seeding the cells we recommend placing the cell medium and the µ-Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.
Trapped air bubbles can be removed from the channel by inclining the µ-Slide and knocking at one edge.

CAUTION – Make sure to avoid uneven incubator shelves and microscope stages. Single cells or cell clusters might roll on one side over time. Please also avoid evaporation and temperature changes. Both will lead to convectional flow.

Exchanging Medium

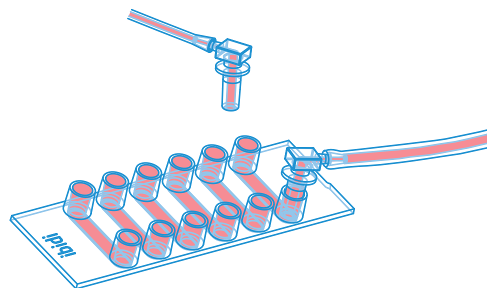
Aspirate both reservoirs and slowly fill 120 µl of fresh medium into one of the reservoirs, which will replace the channel volume by gravity flow.

CAUTION – Carefully remove and re-fill liquid with a standard pipette. Be careful when using a cell culture aspiration device as this may flush away partially attached cells or clusters.

Connecting Tubing for Perfusion

The µ-Slide is fully compatible with the ibidi Pump System and other pump setups.

1. Fill the Luer ports with cell-free medium until they are completely filled. This ensures air bubble-free connection of the tubing.
2. Prepare the perfusion system by 1) filling the tubing completely and 2) pinching off the tubing with a screw clamp or a hose clip.
3. Connect the male Luer ends of the clamped tubing to the Luer ports one at a time. Make sure not to trap air. Remove access culture medium with tissue.



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

For more information about fluidic connections, please also see our Application Note 13 and Application Note 31.

Shear Stress in the µ-Slide VI 0.4

Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11 "Shear stress and shear rates".

The shear stress (τ) in the µ-Slide VI 0.4 can be calculated by inserting the flowrate (Φ) and the dynamical viscosity (η) in the following formula:

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

$$\begin{aligned} Shearstress \quad & \tau \left[\frac{dyn}{cm^2}\right] \\ Dynamicalviscosity \quad & \eta \left[\frac{dyn \cdot s}{cm^2}\right] \\ Flowrate \quad & \Phi \left[\frac{ml}{min}\right] \end{aligned}$$

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

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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, sqr30, pit75, 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”

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 ℃)	444970-9010	220816	03/2023
Zeiss	Immersol 518 F (37 ℃)	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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