

### Instructions

# μ-Slide VI <sup>0.4</sup> μ-Pattern <sup>RGD</sup> Test Patterns 3



The ibidi product family is comprised of a variety of  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

The convenient six channel format of the  $\mu$ -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  $\mu$ -Slide VI  $^{0.4}$  can be connected to a pump and enables you to observe cells under flow conditions.

The  $\mu$ -Patterning technology enables spatially defined cell adhesion for various 2D and 3D cell culture applications. The cell-adhesive patterns are irreversibly printed on the non-adhesive Bioinert surface of the ibidi Polymer Coverslip, allowing for precisely controlled cell adhesion. The  $\mu$ -Patterns are dry-stable, sterile, and ready to use. Bioinert itself is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip No. 1.5. In contrast to standard ultra-low attachment (ULA) coatings, Bioinert is completely non-adherent and allows no binding of any biomolecule, even in long-term experiments.

#### **Overview**

This document is applicable to the following product numbers:

Cat. No.	Product Name
83653	<b>μ-Slide VI 0.4 μ-Pattern RGD Test Patterns 3:</b> #1.5 polymer coverslip, micropatterned surface with RGD motif, 6 patterns, surface passivation with Bioinert, sterilized, individually packed

#### Material

ibidi  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates are made of a polymer that has the highest optical quality. The polymer coverslip on the bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. Also, it is not possible to detach the bottom from the upper part. The  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates are intended for one-time use and are not autoclavable, since they are only temperature-stable up to  $80^{\circ}$ 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 ibidi Polymer Coverslip		
Refractive index n <sub>D</sub> (589 nm)	1.52	
Abbe number	56	
Thickness	No. 1.5 (180 μm)	
Material	Polymer coverslip	

Please note! The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 5.

# **Shipping and Storage**

The  $\mu$ -Slides,  $\mu$ -Dishes and  $\mu$ -Plates are sterilized and welded in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is listed in the following table.

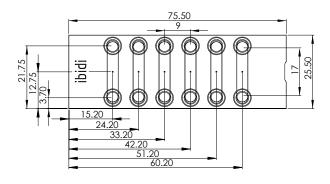
Conditions			
Shipping conditions	Ambient		
Storage conditions	RT (15-25°C)		
Shelf Life			
μ-Patterning	36 months		
	products in a dry place (relative h		

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



# Geometry of the $\mu$ -Slide VI $^{0.4}$

The  $\mu\text{-Slide\,VI}^{0.4}$  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.

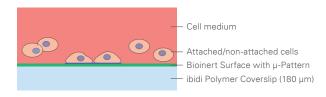


#### Geometry

Geometry		
Outer dimensions in mm $(w \times l)$	25.5 × 75.5	
Adapters	Female Luer	
Number of channels	6	
Channel volume	$30\mu l$	
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	0.6 cm <sup>2</sup> per channel	
Coating area using 30 µl	1.2 cm <sup>2</sup> per channel	
Bottom	ibidi Polymer Coverslip	

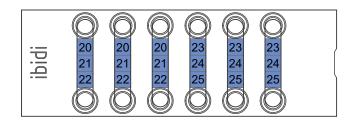
### Geometry of the µ-Pattern

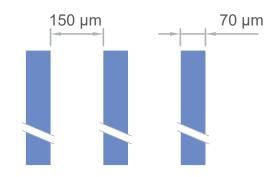
The cell-adhesive patterns are irreversibly printed on the non-adhesive Bioinert surface of the ibidi Polymer Coverslip, allowing for precisely controlled cell adhesion. The patterns are not visible under the microscope.



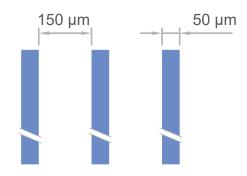
The  $\mu$ -patterned surface presents the covalently bound tripeptide Arg-Gly-Asp (Arginine, Glycine, Aspartate - RGD). This amino acid sequence from the extracellular matrix protein fibronectin mediates cell attachment in many cell types.

The  $\mu$ -Slide VI  $^{0.4}\,\mu$ -Pattern  $^{RGD}$  Test Patterns 3 is a 6 pattern layout with the following geometry:

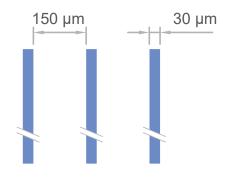




Pattern No. 20

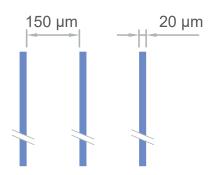


Pattern No. 21

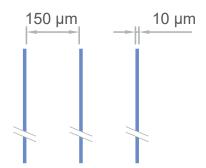


Pattern No. 22

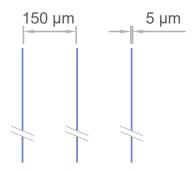




Pattern No. 23



Pattern No. 24



Pattern No. 25

#### Note:

The patterns are separated by 150  $\mu$ m wide  $\mu$ -patterned lines. These separation lines can be used for navigation and for cell adhesion control.

### The Bioinert Surface

The Bioinert surface 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. Unlike with the ibiTreat and Uncoated surfaces, a coating is not possible.

#### **Characteristics of the Bioinert Surface**

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

### **Seeding Cells**

Follow the steps below as a guideline for a general cell application protocol. 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  $1-7 \times 10^5$  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<sub>2</sub> 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.
  Leave each reservoir filled with 60 μl cell-free medium.

### Tip:

The day before seeding the cells we recommend placing the cell medium and the  $\mu$ -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.

# $\mu$ -Slide VI $^{0.4}\,\mu$ -Pattern $^{RGD}$ Test Patterns 3

# **Instructions**

### Tip:

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.

# Tip:

For best results, read our Application Note 65: Cell Adhesion on ibidi  $\mu$ -Patterns: Parameters and Optimization.

### **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.

#### Attention:

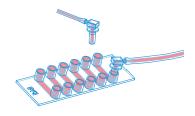
Carefully remove and re-fill liquid with a standard pipette. Be careful when using a cell culture aspiration device as this may flush away partially attached cells or clusters.

### **Connecting Tubing for Perfusion**

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

Detailed information about flow rates, shear stress, and shear rates is provided in Application Note 11 "Shear stress and shear rates". Suitable Tube Adapter Sets are also available (see page 6). They consist of a tubing (20 cm) with inner diameter of 1.6 mm and adapters for the connection between the ibidi  $\mu$ -Slide (female Luer) and the tubing of the pump in use.

- 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.

# Shear Stress in the $\mu$ -Slide VI $^{0.4}$

The shear stress ( $\tau$ ) in the  $\mu$ -Slide VI  $^{0.4}$  can be calculated by inserting the flowrate ( $\Phi$ ) and the dynamical viscosity ( $\eta$ ) in the following formula:

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

 $Shearstress \qquad \tau \left[ \frac{dyn}{cm^2} \right]$   $Dynamical viscosity \qquad \eta \left[ \frac{dyn \cdot s}{cm^2} \right]$   $Flowrate \qquad \Phi \left[ \frac{ml}{min} \right]$ 

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



# $\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ Test Patterns 3

# Instructions

# **Chemical Compatibility**

The following table provides some basic information on the chemical and solvent compatibility:

Chemical / Solvent	Compatibility
Methanol	yes
Ethanol	yes
Formaldehyde	yes
Acetone	yes, without lid
Mineral oil	no
Silicone oil	yes
Immersion oil	See <b>Immersion Oil</b> on page 5.

# **Microscopy**

To analyze your cells, no special preparations are necessary. Cells can be directly observed live or fixed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and storage of fixed and stained samples, ibidi provides mounting media (50001 and 50011) optimized for  $\mu$ -Dishes,  $\mu$ -Slides, and  $\mu$ -Plates.

#### **Immersion Oil**

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	50101	16-12-27	01/2017
Cargille	Туре А	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.	03/2011
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	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012



# **Instructions**

# $\mu\text{-Slide\,VI}$ $^{0.4}\,\mu\text{-Pattern}$ Test Patterns 3

# **Ordering Information**

The  $\mu\text{-Patterning}$  family is available in different slide formats.



Cat. No.	Description	Pcs./Box
83601	$\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ , sqr20, pit110, hex: #1.5 polymer coverslip, micropatterned surface with RGD motif, 20 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	10
83601-S	$\mu$ -Slide VI <sup>0.4</sup> $\mu$ -Pattern <sup>RGD, sqr20, pit110, hex</sup> Trial Pack: #1.5 polymer coverslip, micropatterned surface with RGD motif, 20 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	2
83603	<b>μ-Slide VI</b> <sup>0.4</sup> <b>μ-Pattern</b> <sup>RGD, sqr30, pit110, hex: #1.5 polymer coverslip, micropatterned surface with RGD motif, 30 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed</sup>	10
83603-S	$\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ , sqr30, pit110, hex Trial Pack: #1.5 polymer coverslip, micropatterned surface with RGD motif, 30 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	2
83602	<b>μ-Slide VI</b> <sup>0.4</sup> <b>μ-Pattern</b> <sup>RGD, cir100, pit500, hex: #1.5 polymer coverslip, micropatterned surface with RGD motif, 100 μm circles, 500 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed</sup>	10
83602-S	$\mu$ -Slide VI <sup>0.4</sup> $\mu$ -Pattern <sup>RGD</sup> , cir100, pit500, hex Trial Pack: #1.5 polymer coverslip, micropatterned surface with RGD motif, 100 $\mu$ m circles, 500 $\mu$ m pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	2
83651	$\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ Test Patterns 1: #1.5 polymer coverslip, micropatterned surface with RGD motif, 15 patterns, surface passivation with Bioinert, sterilized, individually packed	2
83652	$\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ Test Patterns 2: #1.5 polymer coverslip, micropatterned surface with RGD motif, 6 patterns, surface passivation with Bioinert, sterilized, individually packed	2
83653	$\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ Test Patterns 3: #1.5 polymer coverslip, micropatterned surface with RGD motif, 6 patterns, surface passivation with Bioinert, sterilized, individually packed	2



# **Instructions**

# $\mu$ -Slide VI $^{0.4}$ $\mu$ -Pattern $^{RGD}$ Test Patterns 3



Cat. No.	Description	Pcs./Box
83801	<b>μ-Slide 8 Well</b> high <b>μ-Pattern</b> RGD, sqr20, pit110, hex: #1.5 polymer coverslip, micropatterned surface with RGD motif, 20 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	10
83801-S	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD, sqr20, pit110, hex Trial Pack: #1.5 polymer coverslip, micropatterned surface with RGD motif, 20 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	2
83803	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD, sqr30, pit110, hex: #1.5 polymer coverslip, micropatterned surface with RGD motif, 30 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	10
83803-S	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD, sqr30, pit110, hex Trial Pack: #1.5 polymer coverslip, micropatterned surface with RGD motif, 30 μm squares, 110 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	2
83802	<b>μ-Slide 8 Well</b> high <b>μ-Pattern</b> RGD, cir100, pit500, hex: #1.5 polymer coverslip, micropatterned surface with RGD motif, 100 μm circles, 500 μm pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	10
83802-S	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD, cir100, pit500, hex Trial Pack: #1.5 polymer coverslip, micropatterned surface with RGD motif, 100 $\mu$ m circles, 500 $\mu$ m pitch, hexagonal layout, surface passivation with Bioinert, sterilized, individually packed	2
83851	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD Test Patterns 1: #1.5 polymer coverslip, micropatterned surface with RGD motif, 15 patterns, surface passivation with Bioinert, sterilized, individually packed	2
83852	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD Test Patterns 2: #1.5 polymer coverslip, micropatterned surface with RGD motif, 6 patterns, surface passivation with Bioinert, sterilized, individually packed	2
83853	$\mu$ -Slide 8 Well high $\mu$ -Pattern RGD Test Patterns 3: #1.5 polymer coverslip, micropatterned surface with RGD motif, 6 patterns, surface passivation with Bioinert, sterilized, individually packed	2

### **Tube Adapter Set**



Cat. No.	Description	Pcs./Box
10831	Tube Adapter Set: sterilized	6x2

# For research use only!

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

© ibidi GmbH, Lochhamer Schlag 11, 82166 Gräfelfing, Germany.