



The ibidi product family is comprised of a variety of  $\mu$ -Slides and  $\mu$ -Dishes, 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  $\mu$ -Slide I Luer I Luer is designed for cell culture under perfusion and all flow applications. Main applications are the simulation of blood vessels for arteriosclerosis research and applying defined shear stress and shear rates on cells inside the channel. The female Luers allow easy connections to tubing and pump systems. The  $\mu$ -Slide I Luer comes in four versions which only differ in their channels' heights and channel volumes.

#### Material

ibidi  $\mu$ -Slides,  $\mu$ -Dishes, and  $\mu$ -Plates are made of a plastic 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 not autoclavable, since they are only temperature-stable up to 80°C/175°F. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the polymer coverslip, which should not be covered.

Optical Properties ibidi I	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	
ibiTreat, Uncoated	36 months	
Collagen IV	18 months	

# Geometry of the $\mu$ -Slide I Luer

The  $\mu\mbox{-Slide}$  I Luer provides standard slide format according to ISO 8037/1.

General Dime	ensions
Outer dimensions	25.5 mm x 75.5 mm
Channel length	50 mm
Channel width	5.0 mm
Volume per reservoir	60 µl
Growth area	2.5 cm <sup>2</sup> per channel
Bottom matches coverslip	No. 1.5

The channel volume depends on the channel height:

Product name	Channel height	Channel volume
µ-Slide I <sup>0.2</sup> Luer	200 µm	50 µl
µ-Slide I <sup>0.4</sup> Luer	400 µm	100 µl
µ-Slide I <sup>0.6</sup> Luer	600 µm	150 µl
µ-Slide I <sup>0.8</sup> Luer	800 µm	200 µl

## Surface

The tissue culture-treated ibiTreat surface is a physical surface modification and optimized for adhesion of most cell types. The uncoated surface is a very hydrophobic surface and allows no direct cell growth. It is suitable for specific coatings or suspension cells.

If you like to establish a particular coating for your demands we recommend testing your coating procedure on uncoated and ibiTreat surfaces, since some proteins and biomolecules adhere differently to hydrophobic or hydrophilic polymer surfaces.

The  $\mu$ -Slide I Luer is also provided with a Collagen Type IV coated surface. Such an adhesion substrate has been shown to stimulate the adhesion and growth of various cell lines. A high quality Collagen IV solution (Corning #356233) is used to pre-coat the  $\mu$ -Slide I Luer.

# Instructions



## Coating

Detailed information about coatings is provided in Application Note 08: Coating protocols for ibidi labware products.

In short, specific coatings are possible following this protocol:

- 1. Prepare your coating solution according to the manufacturer's specifications or reference.
- 2. Apply the channel volume depending on the channel height (see table below) and leave at room temperature for at least 30 minutes.
- 3. Aspirate the solution and wash with the recommended protein dilution buffer.
- 4. The μ-Slide I Luer is ready to be used. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Product Name	Channel Volume	Coating Area
µ-Slide I <sup>0.2</sup> Luer	50 µl	$5.2 \text{ cm}^2$
µ-Slide I <sup>0.4</sup> Luer	100 µl	$5.4 \text{ cm}^2$
µ-Slide I <sup>0.6</sup> Luer	150 µl	$5.6 \text{ cm}^2$
µ-Slide I <sup>0.8</sup> Luer	200 µl	$5.8 \text{ cm}^2$

#### Tip:

You can add the buffer into one channel end and simultaneously aspirate it on the other side.

## Filling and Handling of Channel Slides

In order to avoid air bubbles inside the channels please follow the recommendations below.

When filling the channels put the pipet tip directly to the channel's inlet. Apply the volume with a constant and swift flow.



In special cases, e.g. when the channel surface is hydrophobic or when filling small channels, it might be necessary to fill the channel with a syringe. Use a low volume syringe with 1 or 2.5 ml!



## Important!

When seeding cells, fill only the correct channel volume into the channel. Avoid surplus cell suspension in the reservoirs!

After cell attachment fill 60  $\mu$ l in each well, for a better medium supply to the cells.



## **Medium Exchange**

The following medium exchange protocol is important for cell culture medium exchange, staining, washing and coating procedures.

Empty the reservoirs completely without emptying the channel. Inject the new solution from one side and remove the old solution from the other side. Make sure the old solution is completely replaced. For a 99% exchange add about three times the channel volume from one side.





#### Important!

Take care, that the channel never falls dry during the exchange process. This helps you avoiding air bubbles!

When aspirating the liquid put the pipet tip away from the channel's inlet! This prevents you from evacuating the whole channel.



#### **Cell Culture under Static Conditions**

For many static applications with microscopic imaging, like transfection, immunofluorescence staining or cell morphology the  $\mu$ -Slide I Luer is an optimal solution.

#### Important!

The  $\mu$ -Slide I <sup>0.2</sup> Luer is not recommended for use in static cell culture!

For longer cultivation, a gentle flow is necessary. This can be achieved by a perfusion system or an incubatorcompatible cell culture rocker.

• Trypsinize and count cells as usual. The cell density after seeding strongly depends on the channel's height. We recommend the following cell concentrations and volumes:

Product name	Volume	Cell concentration
µ–Slide I <sup>0.2</sup> Luer	50 µl	$6-14 \ge 10^5 \text{ cells/ml}$
µ–Slide I <sup>0.4</sup> Luer	100 µl	3–7 x 10 <sup>5</sup> cells/ml
µ–Slide I <sup>0.6</sup> Luer	150 µl	$2-4.5 \ge 10^5 \text{ cells/ml}$
µ–Slide I <sup>0.8</sup> Luer	200 µl	$1.5-3.5 \ge 10^5 \text{ cells/ml}$

- Apply the volume directly into the channel. The recommended cell concentration should result in a 50 % optical confluence layer after 24 hours.
- Cover reservoirs with the supplied caps. Incubate at  $37^{\circ}$ C and 5% CO<sub>2</sub> as usual.

• After cell attachment fill each reservoir with 60 µl medium.

Depending on the cells we recommend exchanging the medium every day in static culture: Aspirate both reservoirs (not the channel). Flush fresh medium inside the channel by filling one reservoir with 120  $\mu$ l medium and removing the content of the reservoir from the other well, ensuring the channel is never dry. Leave both reservoirs filled with approx. 60  $\mu$ l each.

#### Tip:

The day before seeding the cells we recommend placing the cell medium, the  $\mu$ -Slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Quick dispensing of cell suspension helps to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.

# **Cell Culture under Flow Conditions**

Due to the Luer adapters,  $\mu$ -Slide I Luer is suitable to any fluidic setup for cell cultivation under flow conditions. Cells are seeded into the channel and the flow is applied after cell attachment.

• Trypsinize and count cells as usual. The cell density after seeding strongly depends on the channel's height. We recommend the following cell concentrations and volumes:

Product name	Volume	Cell concentration
µ–Slide I <sup>0.2</sup> Luer	50 µl	2.5–5 x 10 <sup>6</sup> cells/ml
µ–Slide I <sup>0.4</sup> Luer	100 µl	$1.2-2.5 \ge 10^6 \text{ cells/ml}$
µ–Slide I <sup>0.6</sup> Luer	150 µl	$0.8-1.6 \ge 10^6 \text{ cells/ml}$
µ–Slide I <sup>0.8</sup> Luer	200 µl	$0.6-1.2 \ge 10^6 \text{ cells/ml}$

- Apply the volume directly into the channel. The recommended cell concentration should result in a 100 % optical confluence layer after some hours.
- Cover reservoirs with the supplied caps. Incubate at 37°C and 5 % CO2 as usual.
- After cell attachment fill each reservoir with 60 µl medium.
- The µ-Slide is now ready for applying flow conditions on the adherent cells. Don't trap air bubbles when plugging in the connecting tubes.

## Tip:

The day before seeding the cells we recommend placing the cell medium, the  $\mu$ -Slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Quick dispensing of cell suspension helps to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.

For long term analysis of cells under flow conditions we recommend using  $\mu$ -Slides with ibiTreat surface.

Application Note 13 "HUVECs under perfusion" describes a detailed protocol of a long term experiment with HUVECs and the ibidi Pump System.

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

For connecting several µ-Slides I Luer with each other in a serial way, please refer to our Application Note 25 "Serial Connection of Flow Chamber".

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.



Please contact us for recommended perfusion setups. ibidi provides a variety of channel slides and pump systems.

# Important!

After coating the  $\mu$ -Slide with a coating that must not be dried, seed cells without emptying the channel: First, aspirate all remaining liquid from both reservoirs. Do not empty the channel. Then, fill 100  $\mu$ l cell suspension into one of the reservoirs. After that, slowly remove this volume from the opposite reservoir in order to flush in the cell suspension. Repeat this step until you have brought in three to four times the channel volume for a maximum of cell homogeneity. Make sure to avoid trapped air bubbles.

## 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 a mounting medium (50001) optimized for  $\mu$ -Dishes,  $\mu$ -Slides, and  $\mu$ -Plates.

## **Chemical Compatibility**

The table below provides some basic information on the chemical and solvent compatibility of the  $\mu$ -Slide I Luer. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on ibidi.com.

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.



# Instructions

#### **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
Zeiss	Immersol 518 F	444960	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012
Leica	Immersion Liquid	11513859	n.a.	03/2011
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017

## For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany. © ibidi GmbH, Lochhamer Schlag 11, 82166 Gräfelfing, Germany.

# Instructions

# **Ordering Information**

The  $\mu$ -Slide I Luer is available in different channel heights and surfaces. See table below for choosing your  $\mu$ -Slide I Luer.  $\mu$ -Slide I  $^{0.2}$  Luer

0	Cat. No.	Description
	80166	<b>μ-Slide I</b> <sup>0.2</sup> Luer ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized
	81162	µ-Slide I <sup>0.2</sup> Luer Collagen IV: #1.5 polymer coverslip, sterilized
	81161	$\mu$ -Slide I <sup>0.2</sup> Luer Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized

#### µ-Slide I<sup>0.4</sup> Luer

0	Cat. No.	Description
O E	80176 81172	<b>μ-Slide I</b> <sup>0.4</sup> Luer ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized <b>μ-Slide I</b> <sup>0.4</sup> Luer Collagen IV: #1.5 polymer coverslip, sterilized
	81171	<b>μ-Slide I</b> <sup>0.4</sup> Luer Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized

# µ-Slide I <sup>0.6</sup> Luer

0	Cat. No.	Description
CO.	80186	<b>µ-Slide I</b> <sup>0.6</sup> Luer ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized
	81182	<b>μ-Slide I</b> <sup>0.6</sup> Luer Collagen IV: #1.5 polymer coverslip, sterilized
	81181	$\mu$ -Slide I <sup>0.6</sup> Luer Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized

#### µ-Slide I <sup>0.8</sup> Luer

0	Cat. No.	Description
OF	80196	<b>μ-Slide I</b> <sup>0.8</sup> Luer ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized
	81192 81191	<b>μ-Slide I</b> <sup>0.8</sup> Luer Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized

#### **Tube Adapter Set**

att faces	Cat. No.	Description
E i	10831	Tube Adapter Set: sterilized
J		