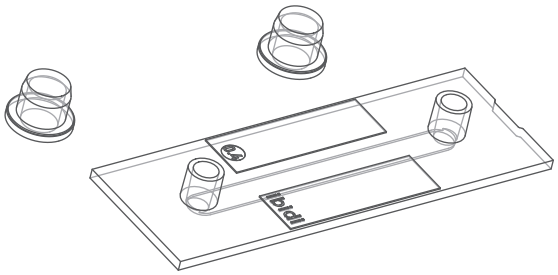
µ-Slide I Luer

Instruction Manual



The ibidi labware comprises 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 recommended microscopy techniques with uncompromised resolution and choice of wavelength. The µ-Slide I Luer is designed for cell culture under perfusion and a range of flow-based applications. The integrated female Luer adapters enable easy connection to tubing and pump systems. This setup allows for the application of defined shear stress and shear rates to cells within the channel.

This document applies to the following products:

80166	**µ-Slide I $^{0.2}$ Luer ibiTreat**
80161	**µ-Slide I $^{0.2}$ Luer Untreated**
80176	**µ-Slide I $^{0.4}$ Luer ibiTreat**
80171	**µ-Slide I $^{0.4}$ Luer Untreated**
80172	**µ-Slide I $^{0.4}$ Luer Collagen IV**
80186	**µ-Slide I $^{0.6}$ Luer ibiTreat**
80181	**µ-Slide I $^{0.6}$ Luer Untreated**
80196	**µ-Slide I $^{0.8}$ Luer ibiTreat**
80191	**µ-Slide I $^{0.8}$ Luer Untreated**

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	
ibiTreat, Untreated	36 months
Collagen IV	18 months

Material

The µ-Slide I Luer 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.

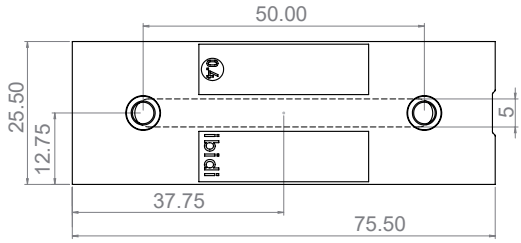
Optical Properties of Polymer Coverslip	
Refractive index (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	Polymer

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".

Geometry

The µ-Slide I Luer provides standard slide format according to ISO 8037/1.

Specifications	
Outer dimensions (w × l)	25.5 × 75.5 mm^2
Channel length	50 mm
Channel width	5.0 mm
Growth area per channel	2.5 cm^2
Volume per reservoir	60 µl
Bottom	ibidi Polymer Coverslip



The µ-Slide I Luer comes in four versions which only differ in their channel heights and volumes.

µ-Slide I Luer	Channel Height (µm)	Channel Volume (µl)
0.2	200	50
0.4	400	100
0.6	600	150
0.8	800	200

Surface

The µ-Slide I Luer is available with either an ibiTreat or an Untreated surface.

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

The hydrophobic Untreated surface of the ibidi Polymer Coverslip offers weak cell adhesion unless pre-coated with an ECM protein. You can apply coatings to the Untreated surface without any restrictions. This surface is suitable for culturing adherent cells that require a specific coating.

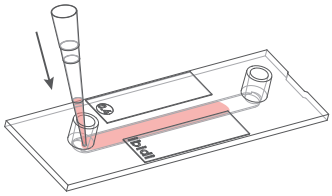
For establishing a particular coating, we advise testing your procedure on both ibiTreat and Untreated surfaces, as proteins and biomolecules may adhere differently to hydrophilic or hydrophobic surfaces.

The µ-Slide I$^{0.4}$ Luer is also provided with a Collagen Type IV-coated surface, which has been demonstrated to enhance the adhesion and growth of various cell lines. A high-quality Collagen IV solution (Corning #356233) is used to pre-coat the µ-Slide I Luer.

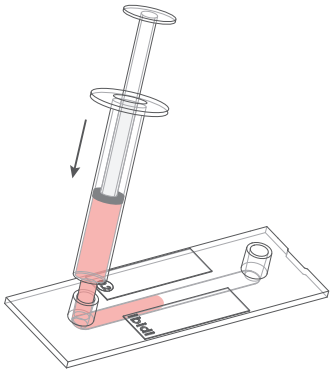
Filling Channels

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.

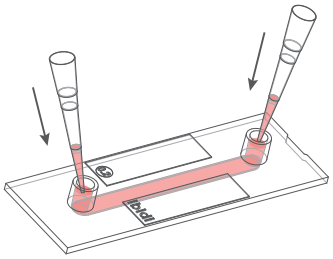


In certain cases, such as when the channel surface is hydrophobic or when filling small channels, it may be necessary to use a syringe. Choose a low-volume syringe with a capacity of 1 or 2.5 ml.



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

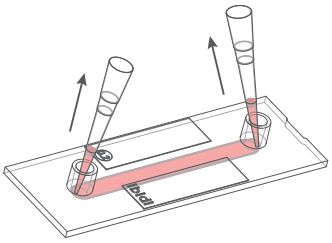
After cell attachment, fill 60 µl cell-free medium into each Luer reservoir as shown. Do not trap air bubbles.



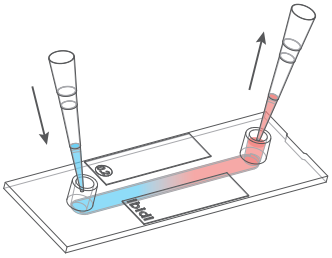
Medium Exchange

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 the respective channel volume of 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 medium 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 avoid air bubbles.

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

Coating

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

In short, specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area and a coating volume per channel as outlined in the following table.

µ-Slide I Luer	Channel Volume (µl)	Coating Area (cm^2)
0.2	50	5.2
0.4	100	5.4
0.6	150	5.6
0.8	200	5.8

2. Apply the respective channel volume into the channel and leave it at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. The coated slide is ready to be used. Be aware that allowing the coated surface to dry out is not recommended, as some coating proteins may degrade upon drying.

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

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

Cell Culture Under Flow

Thanks to the Luer adapters, the µ-Slide I Luer is suitable for cell cultivation under flow conditions. For this, cells are seeded into the channel and the flow is applied after cell attachment.

TIP – For long-term experiments of cells under flow conditions, we recommend using µ-Slides I Luer with ibiTreat surface.

1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. The cell density after seeding strongly depends on the channel height. We recommend the following cell concentrations:

µ-Slide I Luer	Volume (µl)	Cell Conc. (× 10^6 cells/ml)
0.2	50	2.5–5
0.4	100	1.2–2.5
0.6	150	0.8–1.6
0.8	200	0.6–1.2

2. Add the respective volume of cell suspension directly into the channel. Depending on the cell concentration and the specific application, optical confluency can be achieved within a few hours to several days.
3. Cover the reservoirs with the supplied caps. Incubate as usual (e.g., at 37 °C and 5% CO_2).
4. After cell attachment, fill each reservoir with 60 µl medium.
5. The slide is now ready for applying flow conditions on the adherent cells. Do not trap any air bubbles when plugging in the connecting tubes.

Application Note 13: Endothelial Cells Under Perfusion describes a detailed protocol of setting up a long-term flow experiment using HUVECs and the ibidi Pump System in the µ-Slide I Luer.

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 Based on Numerical Calculations.

For a serial connection of several µ-Slides I, please refer to Application Note 25: Serial Connection of Luer-Slides for Flow Experiments.

ibidi provides suitable Tube Adapter Sets (ibidi, 10831). These sets include a 20 cm tubing with an inner diameter of 1.6 mm and adapters for connecting the µ-Slide (female Luer) to the tubing of the used pump.

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

Cell Culture Under Static Conditions

For many static applications that require microscopic imaging, the µ-Slide I Luer is an optimal solution.

CAUTION – The µ-Slide I $^{0.2}$ Luer is not recommended for use in static cell culture. Choose the 0.4, 0.6, or 0.8 versions instead.

1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. The cell density after seeding depends on the channel's height. We recommend the following cell concentrations:

µ-Slide I Luer	Volume (µl)	Cell Conc. (× 10^5 cells/ml)
0.2	50	6–14
0.4	100	3–7
0.6	150	2–4.5
0.8	200	1.5–3.5

2. Add the respective volume of cell suspension directly into the channel. Depending on the cell concentration and the specific application, optical confluency can be achieved within a few hours to several days.
3. Cover the reservoirs with the supplied caps. Incubate as usual (e.g., at 37 °C and 5% CO_2).
4. After cell attachment, 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".

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

Shear Stress Calculations

To calculate the shear stress (τ) in µ-Slide I Luer, insert the flow rate (Φ) and the dynamic viscosity (η) in the formulas provided below:

µ-Slide I $^{0.2}$ Luer: $\tau = \eta \cdot 512.9 \cdot \Phi$

µ-Slide I $^{0.4}$ Luer: $\tau = \eta \cdot 131.6 \cdot \Phi$

µ-Slide I $^{0.6}$ Luer: $\tau = \eta \cdot 60.1 \cdot \Phi$

µ-Slide I $^{0.8}$ Luer: $\tau = \eta \cdot 34.7 \cdot \Phi$

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 I Luer. 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 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.