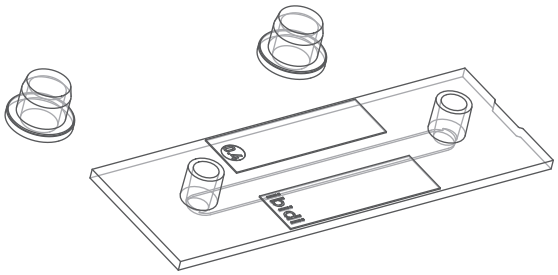
µ-Slide I Luer Glass Bottom

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 glass bottom versions are especially designed for TIRF, super-resolution, and single molecule applications.
The µ-Slide I Luer Glass Bottom 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:

80167	**µ-Slide I $^{0.2}$ Luer Glass Bottom**
80177	**µ-Slide I $^{0.4}$ Luer Glass Bottom**
80187	**µ-Slide I $^{0.6}$ Luer Glass Bottom**
80197	**µ-Slide I $^{0.8}$ Luer Glass Bottom**

Material

The µ-Slide I Luer Glass Bottom is made with a glass coverslip bottom. 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.

CAUTION – Be cautious when handling ibidi labware products with a glass bottom! The glass coverslip or slide is fragile and can break easily. Handle these items carefully to prevent physical injury and damage to devices due to medium leakage.

Optical Properties of Glass Coverslip	
Refractive index	1.523
Abbe number	55
Thickness	No. 1.5H (170 µm ± 5 µm)
Material	Schott borosilicate glass, D 263 M

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	
Glass Bottom	36 months

Surface

The µ-Slide I Luer Glass Bottom is manufactured with a glass coverslip. Washing it (e.g., with PBS) before cell seeding helps remove glass dust, which enhances direct cell growth on the surface.

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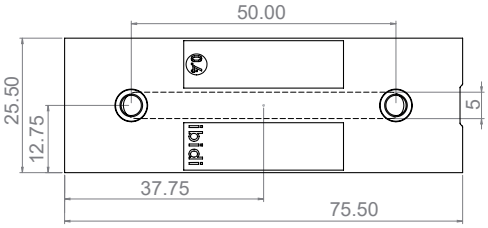
Geometry

The µ-Slide I Luer Glass Bottom 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	Glass Coverslip



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

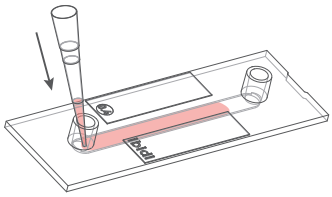
µ-Slide I Luer Glass Bottom	Channel Height (µm)	Channel Volume (µl)
0.2	250	62.5
0.4	450	112.5
0.6	650	162.5
0.8	850	212.5

Please keep in mind that the channel height is formed by the channel height itself (200 µm, ..., 800 µm) plus the thickness of the adhesive layer, which is ca. 50 µm.

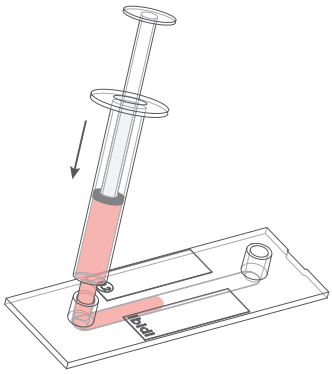
Filling Channels

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.

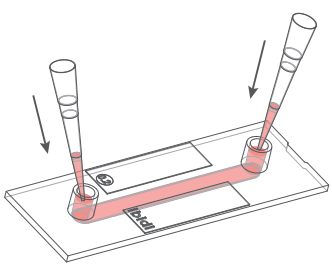


In certain cases, such as when the channel surface is hydrophobic or when filling small channels, it may be necessary to use a syringe. Choose a low-volume syringe with a capacity of 1 or 2.5 ml.



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

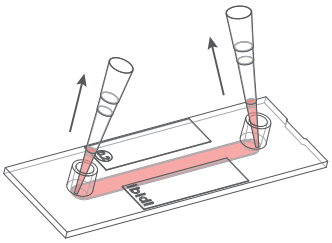
After cell attachment, fill 60 µl cell-free medium into each Luer reservoir as shown. Do not trap air bubbles.



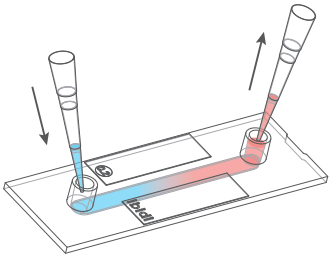
Medium Exchange

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 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 Glass Bottom	Channel Volume (µl)	Coating Area (cm^2)
0.2	62.5	5.2
0.4	112.5	5.4
0.6	162.5	5.6
0.8	212.5	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 Conditions

Thanks to the Luer adapters, µ-Slide I Luer is suitable for any fluidic setup 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 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 Glass Bottom	Volume (µl)	Cell Conc. (× 10^6 cells/ml)
0.2	62.5	2–4
0.4	112.5	1.1–2.2
0.6	162.5	0.7–1.5
0.8	212.5	0.6–1.1

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 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 Luer Glass Bottom with each other, please refer to Application Note 25: Serial Connection of Luer-Slides for Flow Experiments.

We provide 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 Glass Bottom is an optimal solution.

CAUTION – The µ-Slide $I^{0.2}$ Luer Glass Bottom 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 Glass Bottom	Volume (µl)	Cell Conc. (× 10^5 cells/ml)
0.2	62.5	4.8–11.2
0.4	112.5	2.6–6.1
0.6	162.5	1.8–4.1
0.8	212.5	1.4–3.2

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.

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 Glass Bottom, insert the flow rate (Φ) and the dynamic viscosity (η) in the formulas provided below:

µ-Slide I $^{0.2}$ Luer Glass Bottom: $\tau = \eta \cdot 330.4 \cdot \Phi$

µ-Slide I $^{0.4}$ Luer Glass Bottom: $\tau = \eta \cdot 104.7 \cdot \Phi$

µ-Slide I $^{0.6}$ Luer Glass Bottom: $\tau = \eta \cdot 51.5 \cdot \Phi$

µ-Slide I $^{0.8}$ Luer Glass Bottom: $\tau = \eta \cdot 31.0 \cdot \Phi$

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 $\Phi \left[\frac{ml}{min}\right]$

Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the µ-Slide I Luer Glass Bottom. 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	No
Mineral oil	Yes
Silicone oil	Yes
Immersion oil	See Section “Immersion Oil”

Immersion Oil

When using ibidi Glass Bottom products with oil immersion objectives, there is no known incompatibility with any immersion oil on the market. All types of immersion oils can be used.

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

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.
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