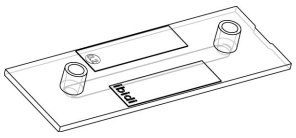


The ibidi product family is comprised of a variety of µ-Slides and µ-Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells.

The glass bottom versions of the µ-Slides and µ-Dishes are especially designed for TIRF, super resolution and single molecule applications.
The µ-Slide I Luer Glass Bottom is a channel slide for cell culture under perfusion and all flow applications. The main applications are applying defined shear stress and shear rates on cells inside the channel such as the simulation of blood vessels for arteriosclerosis research. The female Luers allow easy connections to tubing and pump systems. The µ-Slide I Luer Glass Bottom comes in four versions which only differ in their channels' heights and channel volumes.

Material

The µ-Slide I Luer Glass Bottom is made with a glass coverslip bottom. It is not possible to detach the bottom. The µ-Slide I Luer Glass Bottom is not autoclavable since it is temperature stable only up to 80°C/175°F.

Attention!

Be cautious when handling ibidi labware products with glass bottom! The glass coverslip or glass slide is very fragile and might break easily. Handle with care to avoid physical injury and damage to devices through leakage of the medium.

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

Geometry of the µ-Slide I Luer Glass Bottom

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

General Dimensions	
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^2 per channel
Bottom	Glass Bottom

The channel volume depends on the channel height:

Version	Channel Height	Volume
$^{0.2}$ Luer Glass Bottom	250 µm	62.5 µl
$^{0.4}$ Luer Glass Bottom	450 µm	112.5 µl
$^{0.6}$ Luer Glass Bottom	650 µm	162.5 µl
$^{0.8}$ Luer Glass Bottom	850 µm	212.5 µl

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, ca. 50 µm.

Shipping and Storage

The µ-Slides, µ-Dishes and µ-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	
Glass Bottom	36 months

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 Glass Bottom is ready to be used. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Version	Channel Volume	Coating Area
$^{0.2}$ Luer Glass Bottom	62.5 µl	5.2 cm^2
$^{0.4}$ Luer Glass Bottom	112.5 µl	5.4 cm^2
$^{0.6}$ Luer Glass Bottom	162.5 µl	5.6 cm^2
$^{0.8}$ Luer Glass Bottom	212.5 µl	5.8 cm^2

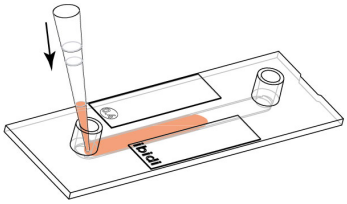
Tip:

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

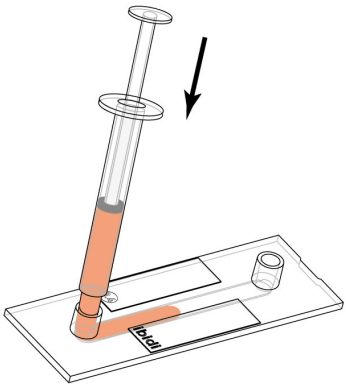
Filling and Handling of Channel Slides

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.



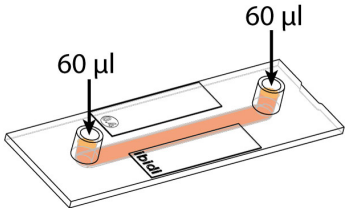
In special cases, e.g. when the channel surface is hydrophobic or when filling small channels, it might be necessary to fill the channel with a syringe. Use a low volume syringe with 1 or 2.5 ml!



Important!

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

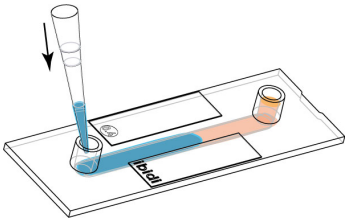
After cell attachment fill 60 µl in each well, for a better medium supply to the cells.



Medium Exchange

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

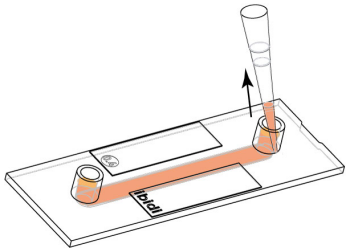
Empty the reservoirs completely without emptying the channel. Inject the new solution from one side and remove the old solution from the other side. Make sure the old solution is completely replaced. For a 99% exchange add about three times the channel volume from one side.



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 µ-Slide I Luer Glass Bottom is an optimal solution.

Important!

The µ-Slide I $^{0.2}$ Luer Glass Bottom 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 incubator-compatible 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
$^{0.2}$ Luer Glass	62.5 µl	4.8–11.2 x 10^5 cells/ml
$^{0.4}$ Luer Glass	112.5 µl	2.6–6.1 x 10^5 cells/ml
$^{0.6}$ Luer Glass	162.5 µl	1.8–4.1 x 10^5 cells/ml
$^{0.8}$ Luer Glass	212.5 µl	1.4–3.2 x 10^5 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°C and 5 % CO_2 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 µl 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 µl each.

Tip:

The day before seeding the cells we recommend placing the cell medium, the µ-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, µ-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
$^{0.2}$ Luer Glass	50 µl	2–4 x 10^6 cells/ml
$^{0.4}$ Luer Glass	100 µl	1.1–2.2 x 10^6 cells/ml
$^{0.6}$ Luer Glass	150 µl	0.7–1.5 x 10^6 cells/ml
$^{0.8}$ Luer Glass	200 µl	0.6–1.1 x 10^6 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 % CO_2 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:

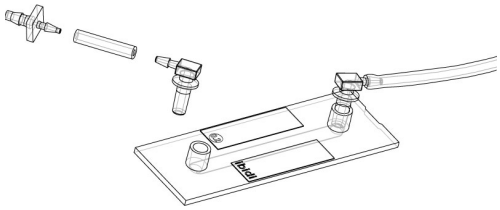
The day before seeding the cells we recommend placing the cell medium, the µ-Slide, and the tubing into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.
Quick dispensing of cell suspension helps to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.

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".

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 µ-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 µ-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 µl 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.

Shear Stress in µ-Slide I Luer Glass Bottom

The shear stress (τ) in µ-Slide I Luer Glass Bottom can be calculated by inserting the flowrate (Φ) and the dynamical viscosity (η) in the following formulas:

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:

$$\tau = \eta \cdot 31.0 \cdot \Phi$$

$$Shearstress \quad \tau \left[\frac{dyn}{cm^2}\right]$$

$$Dynamicalviscosity \quad \eta \left[\frac{dyn \cdot s}{cm^2}\right]$$

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

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

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 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 µ-Dishes, µ-Slides, and µ-Plates.

Chemical Compatibility

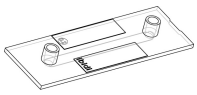
The table below provides some basic information on the chemical and solvent compatibility of the µ-Slide I Luer Glass Bottom. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on ibidi.com.

Chemical / Solvent	Compatibility
Methanol	yes
Ethanol	yes
Formaldehyde	yes
Acetone	no
Mineral oil	yes
Silicone oil	yes
Immersion oil	See **Immersion Oil** on page 4.

Ordering Information

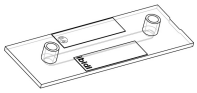
The µ-Slide I Luer family is available in different channel heights and surfaces.

µ-Slide I $^{0.2}$ Luer



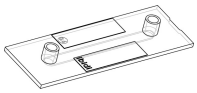
Cat. No.	Description
80166	**µ-Slide I $^{0.2}$ Luer ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
81162	**µ-Slide I $^{0.2}$ Luer Collagen IV**: #1.5 polymer coverslip, sterilized
81161	**µ-Slide I $^{0.2}$ Luer Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
81167	**µ-Slide I $^{0.2}$ Luer Glass Bottom**: #1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

µ-Slide I $^{0.4}$ Luer



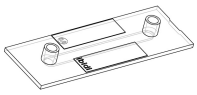
Cat. No.	Description
80176	**µ-Slide I $^{0.4}$ Luer ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
81172	**µ-Slide I $^{0.4}$ Luer Collagen IV**: #1.5 polymer coverslip, sterilized
81171	**µ-Slide I $^{0.4}$ Luer Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
81177	**µ-Slide I $^{0.4}$ Luer Glass Bottom**: #1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

µ-Slide I $^{0.6}$ Luer



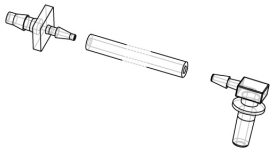
Cat. No.	Description
80186	**µ-Slide I $^{0.6}$ Luer ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
81182	**µ-Slide I $^{0.6}$ Luer Collagen IV**: #1.5 polymer coverslip, sterilized
81181	**µ-Slide I $^{0.6}$ Luer Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
81187	**µ-Slide I $^{0.6}$ Luer Glass Bottom**: #1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

µ-Slide I $^{0.8}$ Luer



Cat. No.	Description
80196	**µ-Slide I $^{0.8}$ Luer ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
81192	**µ-Slide I $^{0.8}$ Luer Collagen IV**: #1.5 polymer coverslip, sterilized
81191	**µ-Slide I $^{0.8}$ Luer Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
81197	**µ-Slide I $^{0.8}$ Luer Glass Bottom**: #1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

Tube Adapter Set



Cat. No.	Description
10831	**Tube Adapter Set**: sterilized

For research use only!

Further information 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.