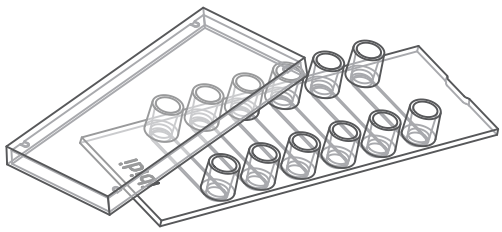
µ-Slide VI $^{0.5}$ Glass Bottom

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 convenient six channel format of the µ-Slide VI $^{0.5}$ Glass Bottom is ideal for static cell cultivation and standard immunofluorescence assays (e.g., for treatment, staining, and microscopy of living or fixed cells). The µ-Slide VI $^{0.5}$ Glass Bottom can also be connected to a pump, enabling cell observation under flow conditions.

This document applies to the following product:

80607	**µ-Slide VI $^{0.5}$ Glass Bottom**

Material

The µ-Slide VI $^{0.5}$ 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.

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

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.

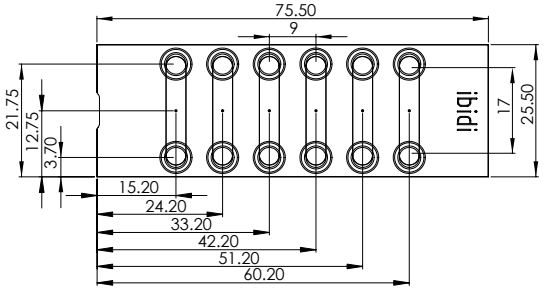
Surface

The µ-Slide VI $^{0.5}$ 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.

Geometry

The µ-Slide VI $^{0.5}$ Glass Bottom provides a standard slide format according to ISO 8037/1. The 9 mm lateral adapter-to-adapter distance (as in 96 well plates) enables the use of multichannel pipets.

Specifications	
Outer dimensions	25.5 × 75.5 mm
Adapters	Female Luer
Number of channels	6
Channel height	0.53 mm
Channel length	17 mm
Channel width	3.8 mm
Height with/without lid	8.8 mm / 7.6 mm
Volume per reservoir	60 µl
Volume of each channel	40 µl
Growth area per channel	0.6 cm^2
Coating area per channel	1.2 cm^2
Bottom	Glass coverslip



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.

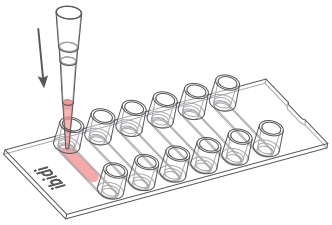
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C)

Shelf Life	
Glass Bottom	36 months

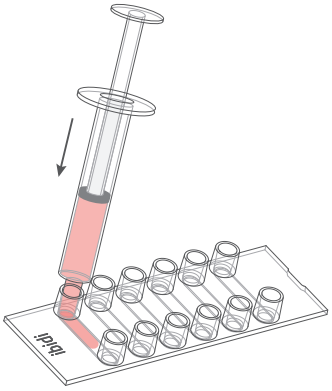
Filling Channels

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.

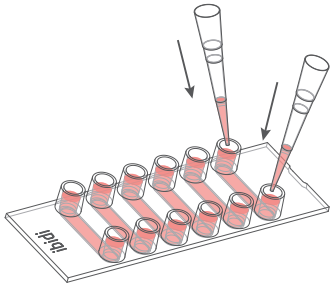


- In certain cases, such as when the channel surface is hydrophobic, it may be necessary to use a syringe. Choose a low-volume syringe with a capacity of 1 or 2.5 ml.



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.



Solution Exchange

The following protocol for continuous solution exchange (e.g., cell culture medium) should be applied for cell culture medium replacement, staining, and washing procedures.

1. Remove the solution 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 120 µl fresh solution 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 solution 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.

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 of 1.2 cm^2 and a volume of 40 µl per channel.
2. Apply 40 µl per 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 – Trapped air bubbles can be removed from the channel by inclining the slide and knocking at one edge.

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

Seeding Cells

1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 2–5 × 10^5 cells/ml suspension should result in a confluent layer within 2–3 days.
2. Add 40 µl cell suspension directly into each channel.

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

TIP – If direct cell seeding is required after coating, seed cells without emptying the channel. For this, replace step 2 with the following steps:

   (a) Directly after coating, aspirate all remaining liquid from both reservoirs. Do not empty the channel.

   (b) Add 120 µl cell suspension into one of the reservoirs.

   (c) Slowly withdraw 120 µl from the opposite reservoir. Make sure to avoid trapped air bubbles.

   (d) Continue with step 3.

3. Cover the slide with the supplied lid and incubate as usual (e.g., at 37 °C and 5% CO_2).
4. After cell attachment, fill each reservoir with 60 µl medium.

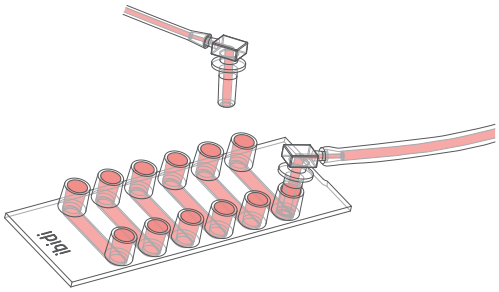
We recommend exchanging the medium every day in static culture, following the protocol in the Section "Solution Exchange".

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

Connecting Tubing for Perfusion

The µ-Slide VI 0.5 Glass Bottom is compatible with the ibidi Pump System and other pump setups for cell cultivation under flow. For this, cells are seeded into the channel and the flow is applied after cell attachment.

1. Fill both Luer ports of the designated flow channel completely with cell-free medium. This ensures air bubble-free connection of the tubing.
2. Prepare the perfusion system: Fill the tubing completely with medium, then pinch it off using a screw clamp or hose clip.
3. Connect the male Luer ends of the clamped tubing to the Luer ports one at a time, ensuring no air is trapped. Remove any excess medium with a tissue.



4. Open the clamped tubing and conduct your perfusion experiment.

For a serial connection of several µ-Slide VI 0.5 Glass Bottom with each other, please refer to Application Note 31: Serial Connection of µ-Slide VI 0.4 Channels for Flow Experiments.

Shear Stress Calculations

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.

To calculate the shear stress (τ) in µ-Slide VI 0.5 Glass Bottom, insert the flow rate (Φ) and the dynamic viscosity (η) in the formula provided below:

$$\tau = \eta \cdot 99.1 \cdot \Phi$$

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

Shear stress	$\tau \left[\frac{dyn}{cm^2}\right]$
Dynamic viscosity	$\eta \left[\frac{dyn \cdot s}{cm^2}\right]$
Flow rate	$\Phi \left[\frac{ml}{min}\right]$

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

Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the µ-Slide VI 0.5 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”

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.