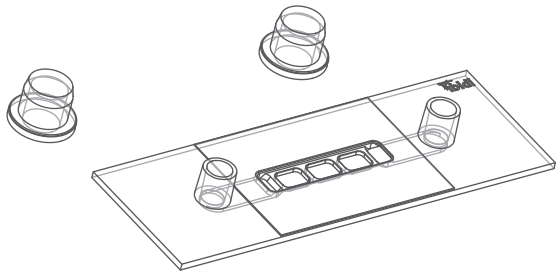
µ-Slide I Luer 3D

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 3D has one channel with three wells for culturing cells on a 3D gel matrix with defined flow. Each well can be filled with a gel, on which cells can be cultivated and microscopically investigated. The channel can be connected to a pump (e.g., to the ibidi Pump System) for the application of defined shear stress.

This document applies to the following products:

87176	**µ-Slide I Luer 3D ibiTreat**
87171	**µ-Slide I Luer 3D Untreated**

Material

The µ-Slide I Luer 3D 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".

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.

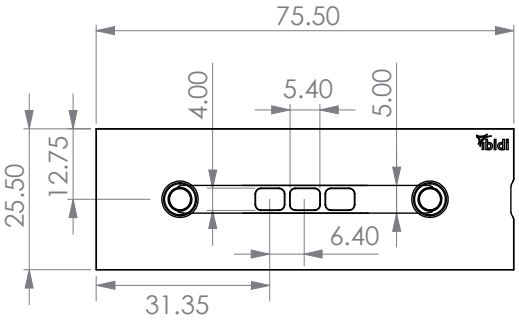
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C)

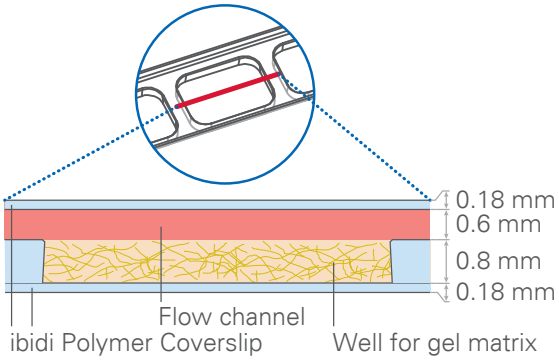
Shelf Life	
ibiTreat, Untreated	36 months

Geometry

The µ-Slide I Luer 3D provides a standard slide format according to ISO 8037/1.

Specifications	
Outer dimensions (w × l)	25.5 × 75.5 mm^2
Number of wells	3
Volume of each well	16 µl
Well dimensions	5.4 mm x 4.0 mm
Well height (without channel)	0.8 mm
Growth area per well	0.21 cm^2
Coating area per well	0.34 cm^2
Channel width	5.0 mm
Channel volume (without wells)	150 µl
Channel height (without well)	0.6 mm
Adapters	Female Luer
Volume per reservoir	60 µl
Top cover	ibidi Polymer Coverslip
Bottom	ibidi Polymer Coverslip





Surface

The µ-Slide I Luer 3D 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.

For assays with a gel matrix inside the wells, both the ibiTreat and the Untreated surface can be directly used. For optimizing the attachment of the gel matrix to the well surface, both surfaces can be optionally precoated with a mediating protein.

TIP – The day before seeding the cells, we recommend placing the cell medium, the slide, and possible tubing for perfusion 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 to avoid trapped air bubbles and leads to maximal homogeneity of cell distribution.

Coating

Non-gel-based coatings are possible when using the µ-Slide I Luer 3D.

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

In short, specific coatings are possible following this protocol:

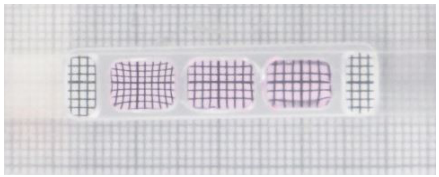
1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 0.34 cm^2 and a volume of 16 µl per well.
2. Apply 16 µl per well and leave it at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended 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.

Gel Volume Optimization

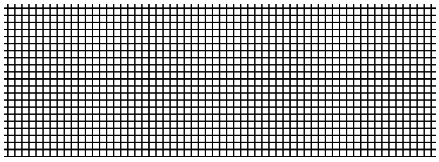
A meniscus-free gel with a plain surface is crucial for optimal imaging. To achieve this, the gel volume of each well needs to be precisely 16 µl. Due to the high viscosity of the gels, the pipet might need to be adjusted to more or less than 16 µl.

To adjust the gel volume accurately, place the slide 1–2 cm above a scale paper (see figure below). Now set your pipet to 16 µl and fill the gel into one well. Observe the markings of the paper through the filled well. If a magnification or demagnification effect is visible, change the volume in ±1 µl steps until you can no longer observe a magnification effect. If the grid of the scale paper appears smaller, the pipetting volume must be increased. If the grid is enlarged, then the pipetting volume must be reduced.

Also, make sure to check again the flatness of the gel surface after polymerization and after filling the channel. Gels can swell or shrink depending on the environmental conditions. Readjust the volume accordingly.



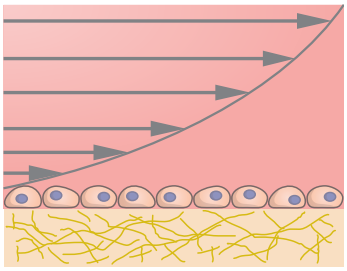
µ-Slide I Luer 3D filled with a Collagen gel. Insufficient gel volume leads to a smaller appearance of the scale paper grid (left well), whereas excessive gel volume leads to an enlarged appearance of the scale paper grid (right well). The middle well is filled with the adequate gel volume and shows no image distortion.



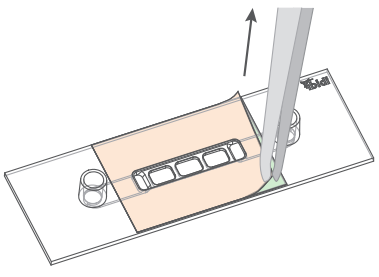
Scale paper for optimizing the gel volume.

Gel-Based Coating and Cell Seeding

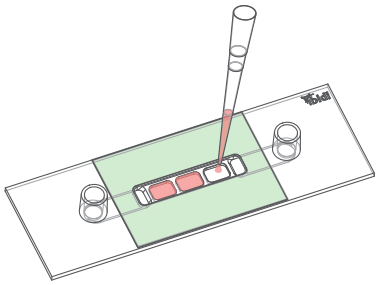
This section describes the standard protocol for seeding adherent cells on top of a gel matrix to apply defined shear stress to a cell monolayer.



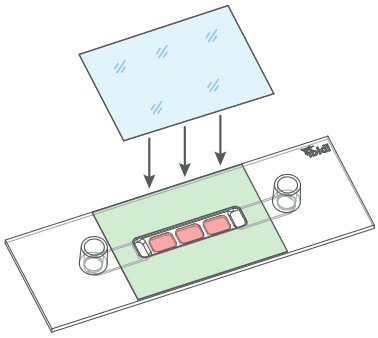
1. Prepare the gel according to the manufacturer's specifications.
2. Remove the protective foil on the upper side of the µ-Slide I Luer 3D.



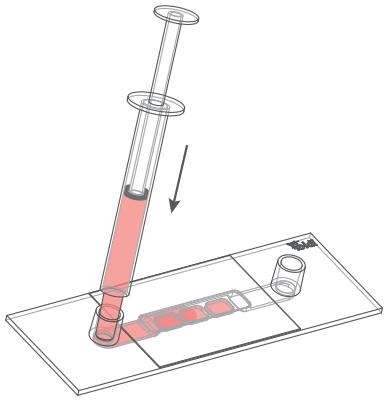
3. Fill each well with 16 µl liquid gel. Avoid air bubbles. Optimize the gel volume as described in the Section “Gel Volume Optimization”.



4. Place the enclosed coverslip on the sticky part of the slide, ensuring the adhesive area between them is tightly sealed. Using your fingers, press down on this area to strengthen the connection.



5. Cover the Luer adapters with the supplied caps.
6. Let the gel polymerize as described in the manufacturer’s specifications.
7. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 2–4.5 × 10^5 cells/ml suspension should result in a confluent layer within 2–3 days. For endothelial cells under flow conditions, we recommend a concentration of 0.8–1.6 × 10^6 cells/ml to achieve 100% optical confluency after cell attachment.
8. Fill a biocompatible 1 ml syringe with ca. 500 µl cell suspension (excess cell suspension helps avoid air bubbles). Connect the syringe directly to the Luer port and apply ca. 250 µl cell suspension into the channel.



9. Remove any remaining cell suspension from the Luer adapters using a standard pipet tip. Be careful not to withdraw the cell suspension from the channel; instead, pipet away from the channel within the Luer adapter.
10. Cover the Luer adapters with the supplied caps.
11. Incubate as usual (e.g., at 37 °C and 5% CO_2) to let the cells attach.
12. After cell attachment, fill each Luer reservoir with 60 µl medium.
13. The slide with the cells on the 3D gel matrix is now ready to be connected to a pump for the application of defined shear stress.

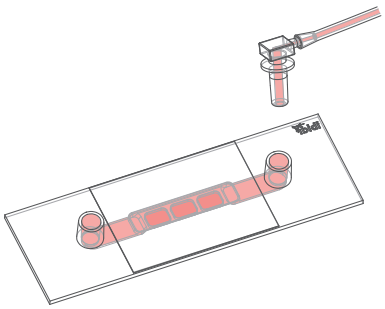
Connecting Tubing for Perfusion

The µ-Slide I Luer 3D is fully compatible with the ibidi Pump System and other pump setups.

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. 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.

1. Fill both Luer ports with cell-free medium until they are completely filled. This ensures air bubble-free connection of the tubing.

2. Prepare the perfusion system by first filling the tubing completely with medium and then pinching off the tubing with a screw clamp or a hose clip.
3. Connect the male Luer ends of the clamped tubing to the Luer ports one at a time. Make sure not to trap air. Remove excess culture medium with a tissue.

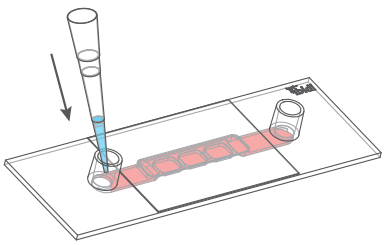


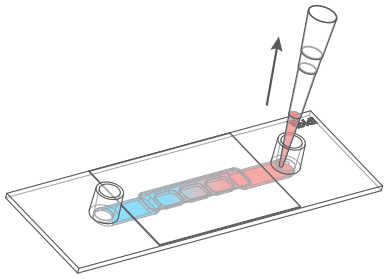
4. Open the clamped tubing and conduct your perfusion experiment.

Medium Exchange

CAUTION – Ensure that the channel does not run dry during the exchange process to prevent the introduction of air bubbles.

1. If the µ-Slide I Luer 3D is connected to a pump, disconnect the tubing from the channel first.
2. 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.
3. Slowly fill 150 µl fresh medium into one of the reservoirs, which will replace the channel volume by gravity flow. Carefully aspirate from the other reservoir using a pipet.





4. Repeat the medium exchange if necessary.
5. Refill the reservoirs using 60 µl medium per reservoir. Before reconnecting the tubing, both Luer ports must be filled completely without any meniscus.
6. Continue with your experiment.

Shear Stress Calculations

To calculate the shear stress (τ) in µ-Slide I Luer 3D, insert the flow rate (Φ) and the dynamic viscosity (η) in the formula provided below:

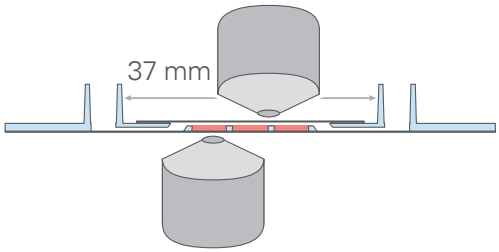
$$\tau = \eta \cdot 60.1 \cdot \Phi$$

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

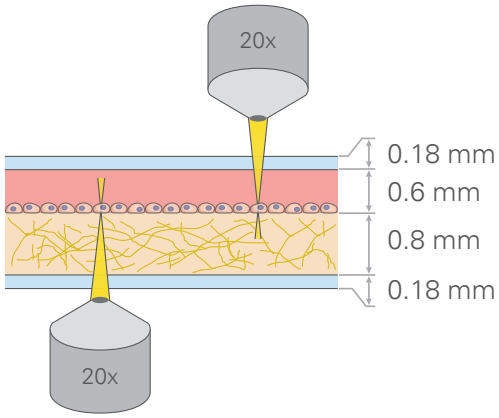
Shear stress	$\tau \left[\frac{dyn}{cm^2}\right]$
Dynamical viscosity	$\eta \left[\frac{dyn \cdot s}{cm^2}\right]$
Flow rate	$\Phi \left[\frac{ml}{min}\right]$

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
The µ-Slide I Luer 3D is partially compatible with upright microscopy. Avoid contact between the Luer adapters and the objective lens. Changing objective lenses may automatically lead to a collision between the objective lens and the Luer adapters.



Please also keep in mind the working distance of the objective lenses. Focusing on cells on the gel matrix requires a minimum working distance of approximately 0.6 mm with upright objective lenses and approximately 0.8 mm with inverted objective lenses.



Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the µ-Slide I Luer 3D. 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	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 ℃)	444970-9010	220816	03/2023
Zeiss	Immersol 518 F (37 ℃)	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

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.