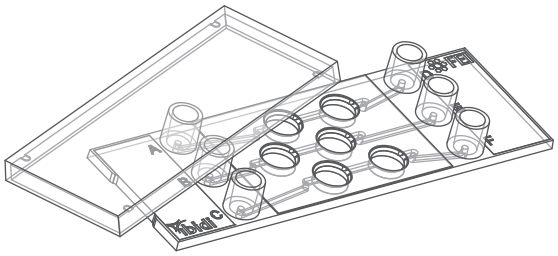
µ-Slide III 3D Perfusion

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 III 3D Perfusion contains six wells, in which cells can be cultivated in 3D matrices and imaged using high-resolution microscopy. Two of the six wells, respectively, are connected by a channel. For optimal nutrition of the cells during long-term culture, the channels can be connected to a pump (e.g., the ibidi Pump System) for perfusing the wells.

This document applies to the following products:

80378	**µ-Slide III 3D Perfusion ibiTreat**
80371	**µ-Slide III 3D Perfusion Untreated**

Material

The µ-Slide III 3D Perfusion 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.

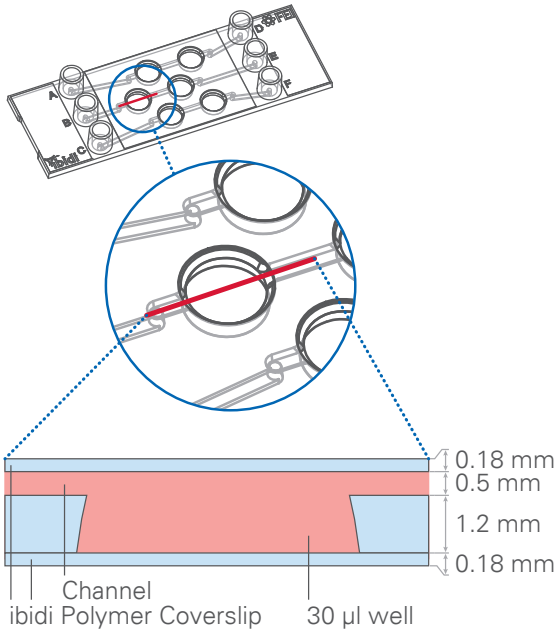
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C)

Shelf Life	
ibiTreat, Untreated	36 months

Geometry

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

Specifications	
Outer dimensions	25.5 × 75.5 mm^2
Number of wells	6
Volume of wells	30 µl
Well diameter	5.5 mm
Well height (without channel)	1.2 mm
Well height (with channel)	1.7 mm
Growth area per well	25 mm^2
Number of channels	3
Total channel volume	130 µl
Channel width	1.0 mm
Adapters	Female Luer
Volume per reservoir	60 µl
Coating area using 30 µl	0.29 cm^2 per well
Coating area using 130 µl	2.4 cm^2 per channel
Top cover/bottom	No. 1.5 ibidi Polymer Coverslip



Surface

The µ-Slide III 3D Perfusion 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.

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. The packaging of the µ-Slide is made of a gas-permeable material, so unpacking is not necessary for the gas equilibration step.

Coating

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

In short, specific coatings are possible following these protocols:

Single Well Coating

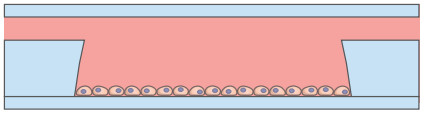
1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 0.29 cm^2 and a volume of 30 µl per well.
2. Apply 30 µl per well and leave it at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. Remove the protection foil on both the upper side of the slide and the enclosed coverslip. Place the 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. 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.

Entire Channel Coating

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 2.4 cm^2 and a volume of 130 µl per channel.
2. Remove the protection foil on both the upper side of the slide and the enclosed coverslip. Place the 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.
3. Apply 130 µl coating solution per channel and leave the slide at room temperature for at least 30 minutes.
4. Aspirate the solution and wash with the recommended protein dilution buffer. You can add the buffer into one channel end and simultaneously aspirate it on the other side.
5. 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.

Seeding Cells in 2D (Standard Protocol)

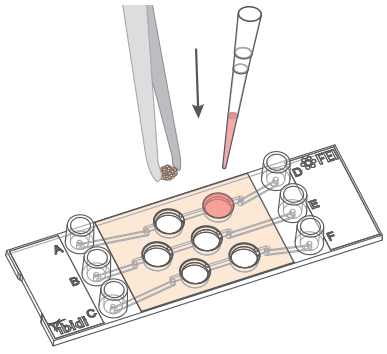
You can use the µ-Slide III 3D Perfusion to culture cells in 2D without any gel matrix.



1. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 0.7–1.7 × 10^5 cells/ml suspension should result in a confluent layer within 2–3 days.
2. Apply 30 µl cell suspension into each well of the µ-Slide III 3D Perfusion. Avoid shaking as this will result in inhomogeneous distribution of the cells.

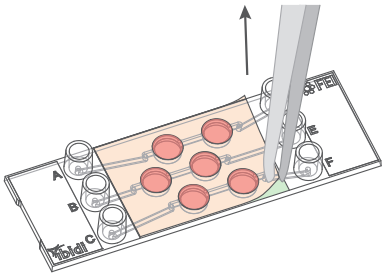
Optional: Direct Sample Insertion Into Wells

The µ-Slide III 3D Perfusion technology allows for the insertion of samples (e.g., cell clusters, which cannot easily be pipetted, such as spheroids or tissue samples), before the enclosed coverslip and the slide are assembled. Place the sample into the well and fill the well with cell-free medium. Then, continue with the standard protocol, starting with step 4.

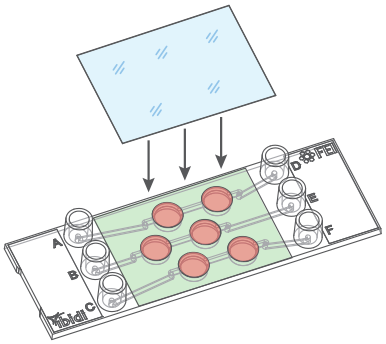


3. Cover the slide with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2) until the cells are attached.

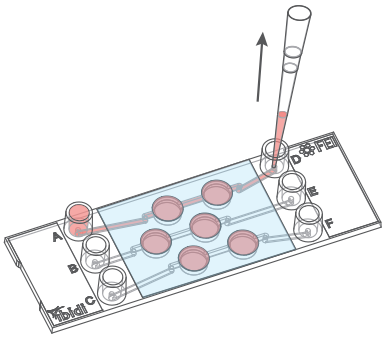
4. Remove the protective foil on the upper side of the slide.



5. Remove the protection foil on the enclosed coverslip. Place the 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.



6. Slowly fill each channel with 70 µl cell-free medium. This helps flush any air out of the channels. Then, fill each Luer reservoir with 60 µl cell-free medium.



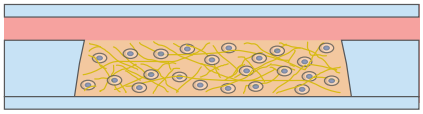
7. Cover the slide with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2).

Insensitive cells can be left in their seeding medium for several days and grow to confluence there. However, optimal results might be achieved when the medium is changed every 1–2 days. For this, carefully aspirate the old medium and replace it by up to 130 µl fresh medium per channel.

3D Cell Culture Applications

The µ-Slide III 3D Perfusion can be used for the following 3D cell culture assays:

Single cells in 3D matrix: cells are embedded in 3D in a gel matrix (e.g., collagen).



1. Prepare your gel matrix according to the manufacturer's specifications. Prepare your cell suspension as usual.

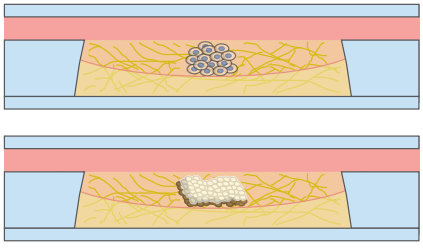
2. Add the cells to the gel matrix to get a final concentration of 2–4 × 10^5 cells/ml. Mix well.

3. Add 30 µl of the mixture to each well of the µ-Slide III 3D Perfusion, close the lid and let the gel polymerize as described in the manufacturer's specifications.

4. Continue with the standard protocol, starting with step 4.

Step-by-step protocols for the preparation of Collagen I gels can be found in Application Note 26: Preparation of Collagen I Gels.

Gel sandwich assay: spheroids or tissue samples are embedded between two gel layers in a sandwich structure.

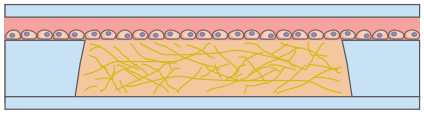


1. Prepare your gel matrix according to the manufacturer's specifications. Apply 15 µl gel matrix into each well of the µ-Slide III 3D Perfusion and let the gel polymerize as described in the manufacturer's specifications.

2. On top of the polymerized gel, add another 15 µl of gel together with the sample to each well and let the gel polymerize as described in the manufacturer's specifications.

3. Continue with the standard protocol, starting with step 4.

Adherent cells seeded on soft matrix: adherent cells are cultured in a channel with a soft gel matrix bottom.



1. Prepare your gel matrix according to the manufacturer's specifications. Apply 30 µl gel matrix into each well of the µ-Slide III 3D Perfusion and let the gel polymerize as described in the manufacturer's specifications.

2. Seal the top of the slide as shown in steps 4–5 of the standard protocol.

3. Prepare your cell suspension with the desired concentration. Depending on your cell type, application of a 2.5–6 × 10^5 cells/ml suspension should result in a confluent layer within 2–3 days.

4. Slowly add 70 µl cell suspension to each channel.

5. After cell attachment, fill each Luer reservoir with 60 µl cell-free medium.

6. Cover the slide with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2).

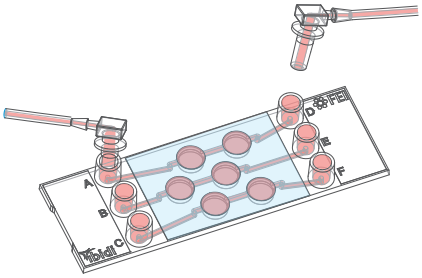
Connecting Tubing for Perfusion

The µ-Slide III 3D Perfusion is compatible with the ibidi Pump System and other pump setups. Please keep in mind that the wells and the channels have been designed for a general perfusion (e.g., for optimal nutrition of the cells during long-term culture). The geometry is not suitable for applying defined and homogeneous shear stress.

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. A flow rate of ca. 0.5–1 ml/min is recommended for optimal supply of oxygen and nutrients.

TIP – For combining the µ-Slide III 3D Perfusion with the ibidi Pump System, we recommend using the Perfusion Set BLUE (ibidi, 10961) with a pressure of ca. 10 mbar. Adjust the pressure to create the desired flow rate for long-term perfusion experiments. Increase the pressure to approximately 15 mbar in case all three channels are connected serially.

Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the µ-Slide III 3D Perfusion. 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”

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

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

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.