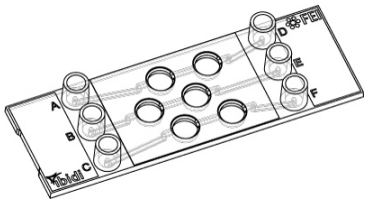


The ibidi product family is comprised of a variety of µ-Slides, µ-Dishes, and µ-Plates which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

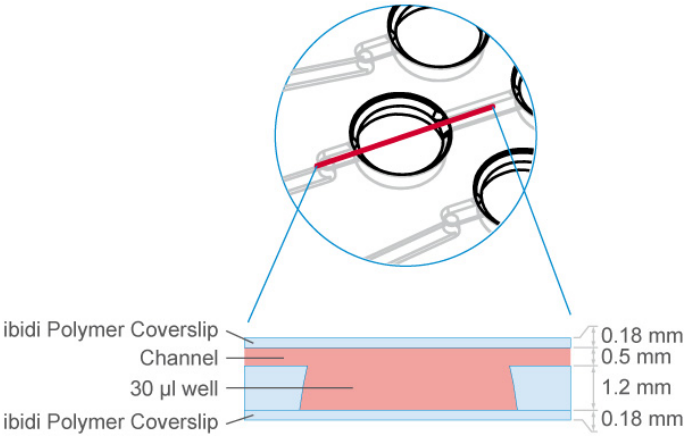
The µ-Slide III 3D Perfusion is an array of 6 wells where cells can be cultivated and, subsequently, investigated with microscopical methods. Two of the 6 wells respectively are connected by a channel. The channels can be connected to a pump for perfusing the wells in order to perform long term cell culture assays with 3D structures.

Material

ibidi µ-Slides, µ-Dishes, and µ-Plates are made of a polymer that has the highest optical quality. The polymer coverslip on the bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. Also, it is not possible to detach the bottom from the upper part. The µ-Slides, µ-Dishes, and µ-Plates are intended for one-time use and are not autoclavable, since they are 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 ibidi Polymer Coverslip	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	Polymer coverslip

Please note! The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 4.



Geometry

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

Geometry of µ-Slide III 3D Perfusion	
Outer dimensions	25.5 mm x 75.5 mm
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	No. 1.5 ibidi Polymer Coverslip
Bottom	No. 1.5 ibidi Polymer Coverslip

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	
ibiTreat, Uncoated	36 months

Coating your µ-Slide III 3D Perfusion

The uncoated µ-Slide version is manufactured from a hydrophobic polymer material. For the cultivation of most cell lines, it is indispensable to treat the uncoated µ-Slide with biopolymers, which mediate cell adhesion and growth, e.g. Collagen, Fibronectin, Poly-L-Lysin, or Poly-D-Lysin. In short, specific coatings are possible following this protocol:

Option 1: Coating the Single Wells

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply 30 µl per well and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with the recommended protein dilution buffer.
- Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

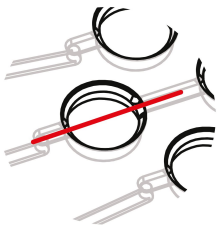
Option 2: Coating the Entire System

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Close the wells with the enclosed polymer coverslip. Therefore, remove the protective foil on the slide and on the polymer coverslip and attach the coverslip on the sticky part of the slide.
- Apply 130 µl per channel and leave at room temperature for at least 30 minutes.
- 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.
- Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

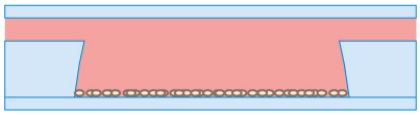
Detailed information about coatings is provided in Application Note 08 "Cell culture coating".

Seeding Cells (Standard Protocol)

There are several possibilities to seed cells into the µ-Slide III 3D Perfusion. This section describes the standard protocol, seeding adherent cells without any gel matrix.

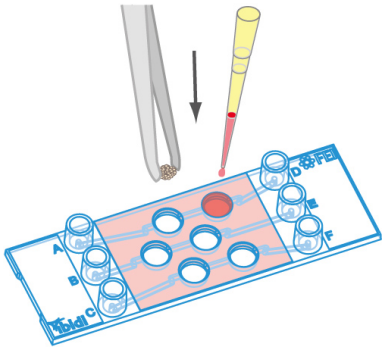


Cross section of one well.



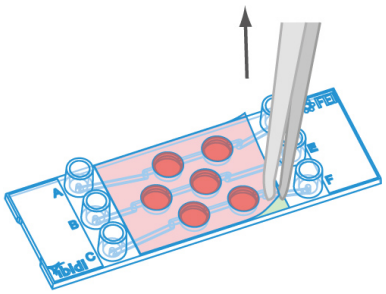
1. Prepare your cell suspension with the desired concentration. Depending on your cell type, application of a 0.7–1.7× 10^5 cells/ml suspension should result in a confluent layer within 2-3 days.

2. Add 30 µl cell suspension into each well of the precoated µ-Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells. - Optionally, place your sample into the wells as shown.

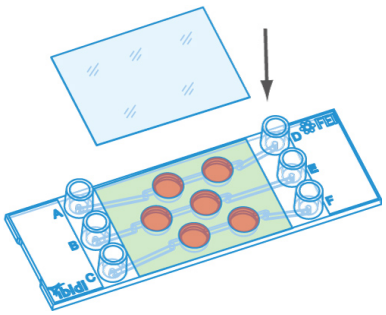


3. Cover the slide with the supplied lid. Incubate at 37°C and 5% CO_2 and wait for cell attachement.

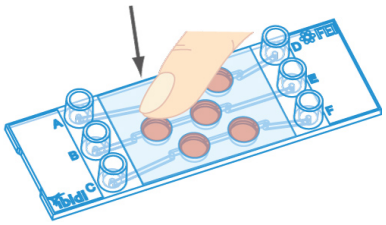
4. Seal the top with the enclosed polymer coverslip: Remove the protective foil on the upper side of the slide.



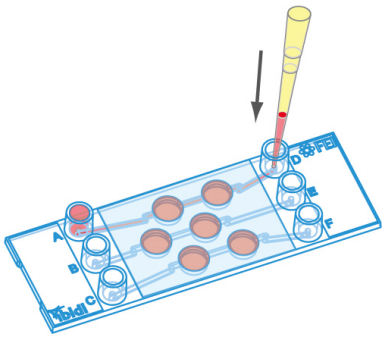
5. Remove the protective foil of the polymer coverslip and place the coverslip on the sticky part of the slide.



6. Make sure the area between the wells is tightly sealed. Therefore press on that area to tighten the connection.



7. Fill the channels slowly with 70 µl cell free medium each, to flush the air out of the channels. Then, fill each Luer adapter with 60 µl cell free medium.



Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. However, best results are achieved when the medium is changed every 1–2 days. Carefully aspirate the old medium and replace it by 130 µl/channel fresh medium.

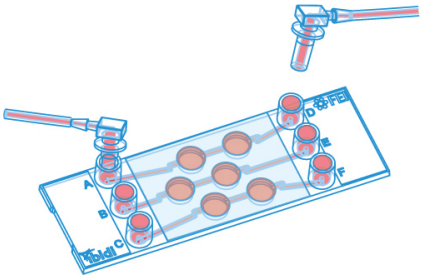
Tip:

The day before seeding the cells we recommend placing the cell medium and the µ-Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

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 are designed for a general perfusion. The geometry is not suitable for creating a defined and homogeneous shear stress.

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



3. A flow rate of ca. 0.5–1 ml/min is recommended.

Tip:

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

Tip:

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

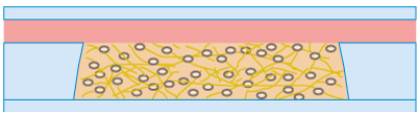
Further Applications

Apart from the standard protocol, the µ-Slide III 3D Perfusion offers more application possibilities.

Single cells in 3D matrix

Cells are captured in 3D in a gel matrix, e.g. Collagen.

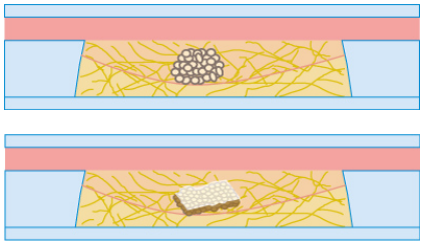
Collagen gel protocols can be found in Application Note 23 "Collagen I Gel for 3D Cell Culture".



1. Prepare your cell suspension.
2. Mix cells and gel matrix well to a final concentration of 2–4 × 10^5 cells/ml.
3. Add 30 µl of the mixture to the wells and wait for polymerization.
4. Continue with step 4 of the standard protocol.

Gel sandwich assay

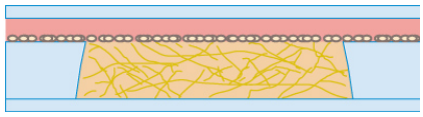
Spheroids or tissue samples are embedded between two layers of gel in a sandwich structure.



1. Add 15 µl gel matrix into each well of the µ-Slide.
2. After polymerization, fill each well with additional 15 µl gel, together with the sample and wait for polymerization.
3. Continue with step 4 of the standard protocol.

Adherent cells seeded on soft matrix

Adherent cells are cultured in a channel with a soft gel matrix bottom.



1. Add 30 µl gel matrix into each well of the µ-Slide and wait for polymerization.
2. Seal the top of the µ-Slide as shown in step 4–6 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 reservoir with 60 µl cell free medium.
6. Continue with step 3 of the standard protocol.

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 mounting media (50001 and 50011) optimized for µ-Dishes, µ-Slides, and µ-Plates.

Chemical Compatibility

The following table provides some 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, please visit the FAQ section on ibidi.com.

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

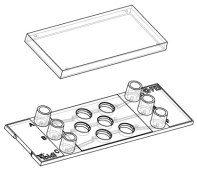
Immersion Oil

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 as non-compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil	50101	16-12-27	01/2017
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.	03/2011
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	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012

Ordering Information

The µ-Slide III 3D Perfusion is available in two product versions.



Cat. No.	Description
80371	**µ-Slide III 3D Perfusion Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
80376	**µ-Slide III 3D Perfusion ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized

For research use only!

Further information can be found at ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0.