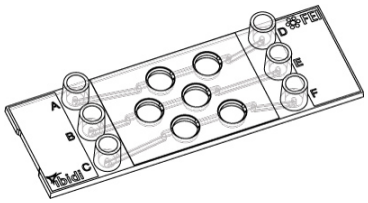


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised 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 plastic 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 not autoclavable, since they are only temperature–stable up to 80°C/175°F. Please note that gas exchange between the medium and 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.

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 of Different Surfaces	
ibiTreat, Glass Bottom, ESS	36 months
Collagen, Poly-L-Lysine	18 months

Geometry

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

Geometry of µ–Slide III 3D Perfusion	
Number of wells	6
Volume of wells	30 µl
Well diameter	5.5 mm
Well depth	1.2 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 130 µl	2.4 cm^2 per channel
Bottom matches coverslip	No. 1.5
Top cover matches coverslip	No. 1.5

Coating your µ–Slide III 3D Perfusion

The uncoated µ–Slide is manufactured from hydrophobic plastic. For the cultivation of most cell lines, it is indispensable to treat the uncoated µ–Slide with biopolymers, which mediate cell adhesion and growth, eg. Collagen IV, Fibronectin, Poly–L–Lysin, and Poly–D–Lysin.

Option 1:

- 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!

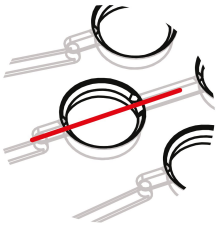
Option 2:

- 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!

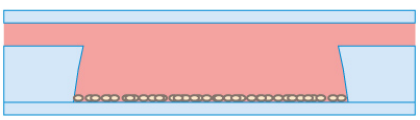
Detailed information about coatings is provided in Application Note 08 "Cell culture coating".

Seeding Cells (Standard Protocol)

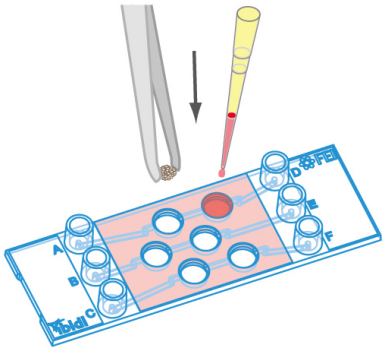
There are several possibilities to seed cells into the µ–Slide III 3D Perfusion. This section describes the standard protocol, seeding adherent cells without any gel matrix.



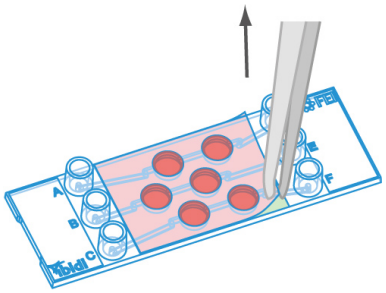
Cross section of one well.



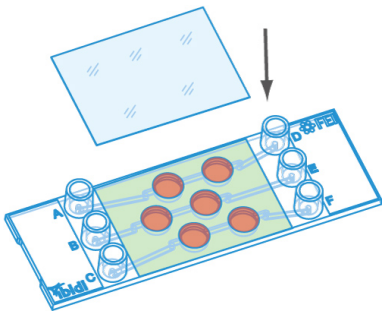
1. Prepare your cell suspension with the desired concentration. Depending on your cell type, application of a 0.7-1.7× 10^5 cells/ml suspension should result in a confluent layer within 2-3 days.
2. Add 30 µl cell suspension into each well of the precoated µ–Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells.



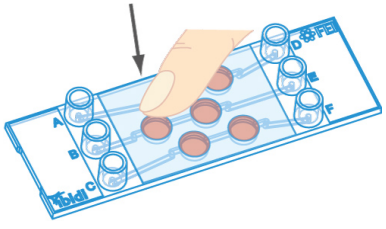
3. Cover the slide with the supplied lid. Incubate at 37°C and 5% CO_2 and wait for cell attachement.
4. Seal the top with the enclosed polymer coverslip: Remove the protective foil on the upper side of the slide.



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

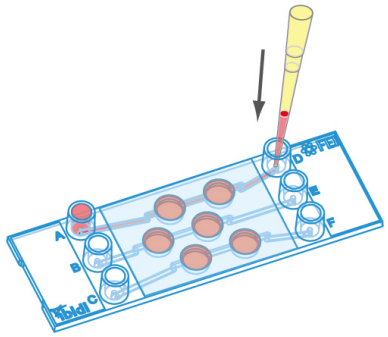


6. Make sure the area between the wells is tightly sealed. Therefore press on that area to tighten the connection.

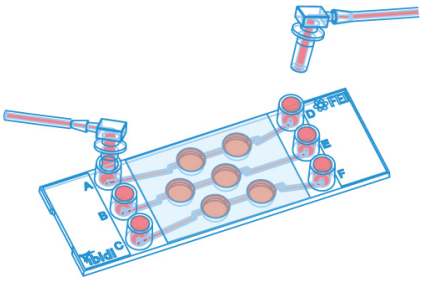


7. Fill the channels slowly with 70 µl cell free medium

each, to flush the air out of the channels. Then, fill each reservoir with 60 µl cell free medium.



8. For flow applications connect the reservoirs with Luer adapters. A flow of <1 ml/min is recommended.



Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. However, best results might be achieved when the medium is changed every 1-2 days. Carefully aspirate the old medium and replace it by 130 µl/channel fresh medium. The volume of a single well is very small. Depending on your cell type the medium might be consumed after some hours. If you want to incubate your cells for longer than a couple of hours we recommend to aspirate and refill cell medium every day.

Tip:

If the wells are properly filled with 30 µl, the liquid surface is planar and in good alignment with the µ–Slides surface. This is how you will be able to observe the whole well area with unimpaired phase contrast.

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.

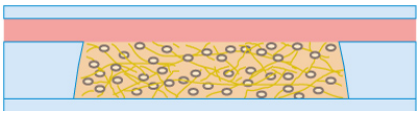
Further Applications

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.

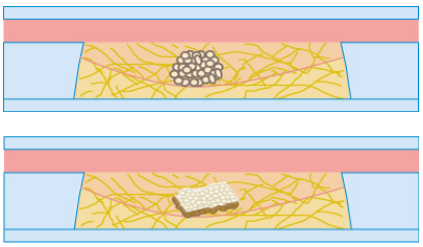
Collagen gel protocols can be found in Application Note 23 "Collagen I Gel for 3D Cell Culture".



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

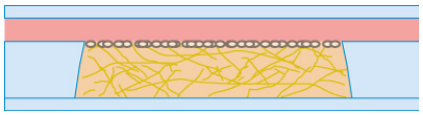
Spheroids or tissue samples are embedded between two layers of gel in a sandwich structure.



1. Add 15 µl gel matrix into each well of the µ–Slide.
2. After gelation, 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 8 of the standard protocol.

Preparation for Cell Microscopy

To analyze your cells, no special preparations are necessary. Cells can be observed live, or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide material is compatible with a variety of chemicals, e.g., acetone or methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180 µm, high resolution microscopy is possible.

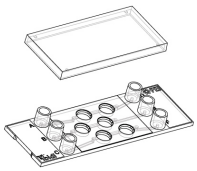
Immersion Oil

When using oil immersion objectives, use only the immersion oils specified in the table. The use of a non–recommended oil could damage the plastic material and the objective.

Company	Product	Ordering Number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

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 technical specifications 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. All products are developed and produced in Germany.