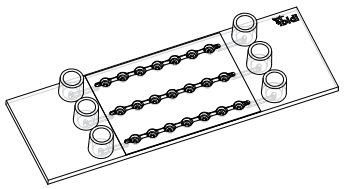


The ibidi product family is comprised of a variety of µ-Slides and µ-Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised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

The µ-Slide Spheroid Perfusion is a specialized flow chamber for 3D aggregates. A special well geometry ensures optimal nutrition and gas diffusion for spheroids, organoids or tissue throughout the experimental time. In combination with perfusion, a maximal viability and proliferation with a minimum of mechanical shear stress can be achieved.

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.

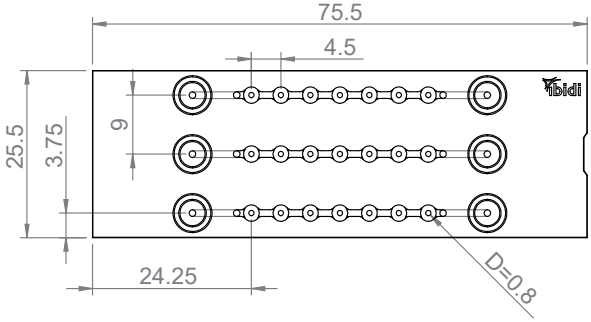
Optical Properties ibidi Polymer Coverslip	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	Polymer coverslip

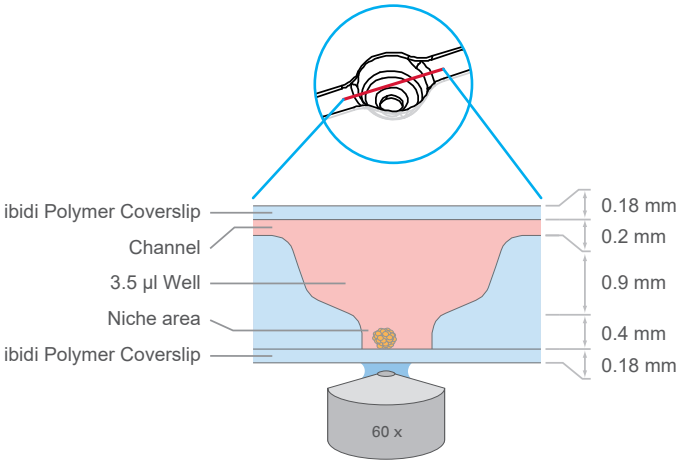
Please note! The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 7.

Geometry of the µ-Slide Spheroid Perfusion	
Outer dimensions	25.5 mm x 75.5 mm
Number of channels	3
Number of wells	3 × 7
Volume per well	3.5 µl
Well height (bottom niche)	0.4 mm
Well height (total)	1.3 mm
Well diameter (bottom)	0.8 mm
Channel volume (total)	45 µl
Channel height	0.2 mm
Channel width	1.0 mm
Growth area per well	0.5 mm^2
Coating area per well	9.7 mm^2
Adapters	Female Luer
Volume per reservoir	60 µl
Top cover	ibidi Polymer Coverslip
Bottom	ibidi Polymer Coverslip

Geometry

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





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
Bioinert, µ-Patterning	36 months

Store the Bioinert and the µ-Patterning products in a dry place (relative humidity <50%).

Surface

Bioinert is the most recommended surface for spheroids or suspension cells. Unlike ibiTreat and Uncoated, the Bioinert surface cannot bind any proteins so cell adhesion is impossible.
The tissue culture-treated ibiTreat surface is a physical surface modification and optimized for adhesion of most cell types. ibiTreat is a hydrophilic surface.
Uncoated is a very hydrophobic surface and allows no direct cell growth. It is suitable for specific coatings or suspension cells.
If you like to establish a particular coating for your demands we recommend testing your coating procedure on the Uncoated and ibiTreat surfaces, since some proteins and biomolecules adhere differently to hydrophobic or hydrophilic polymer surfaces. The Bioinert surface cannot be coated with proteins.

Coating

Detailed information about coatings is provided in Application Note 08: Coating protocols for ibidi labware products.

In short, specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications or reference.
2. Apply 3.5 µl per well and leave at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. The µ-Slide Spheroid Perfusion is ready to be used. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

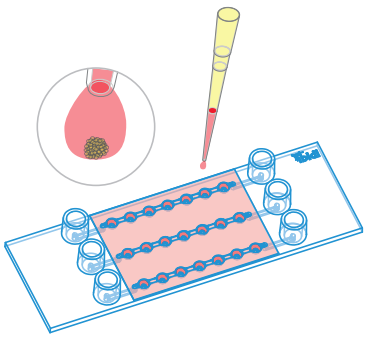
Note:

The Bioinert surface cannot be coated with proteins.

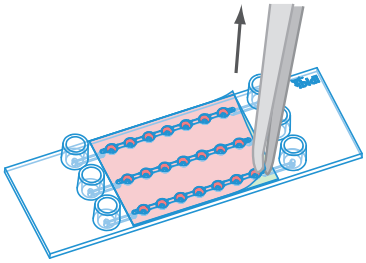
Transfer of Spheroids

Cell aggregates, like spheroids/organoids can be transferred into the single wells of the µ-Slide Spheroid Perfusion following the protocol below. The Bioinert surface is most recommended for this application.

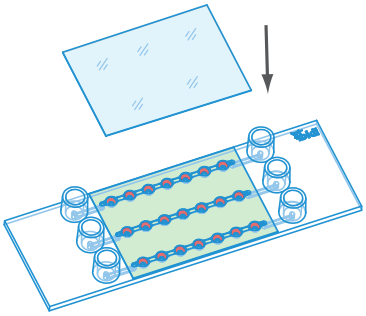
1. Prepare spheroids using your preferred method. Make sure the aggregates have enough stability to be transferred.
2. Prepare 20 µl pipet tips which allow free passage for your spheroids. If necessary, cut the tip with a scalpel to widen the tip's diameter.
3. Per well, transfer one spheroid inside a medium droplet. Use a pipette tip with an opening wide enough for contact-free passage of the spheroid. Make sure not to overfill the single wells.



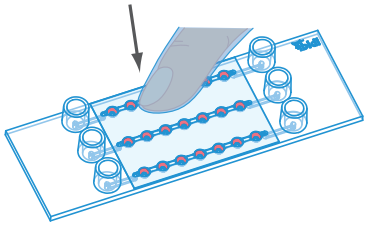
4. Control the integrity of the spheroids under the microscope.
5. Remove the protective foil from the upper side of the µ-Slide. Tweezers are very convenient for this step.



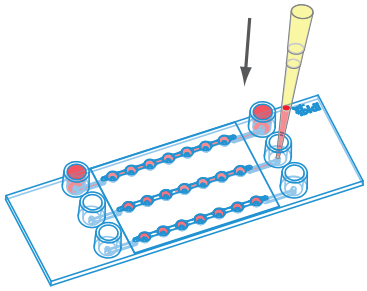
6. Remove the protective foil from the polymer coverslip and place the coverslip on the sticky upper side of the µ-Slide.



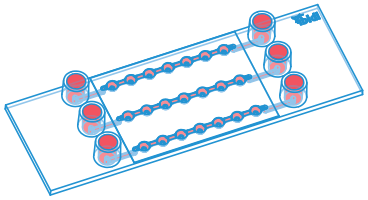
7. Make sure the area between the wells is tightly sealed. Therefore press on that area to tighten the connection.



8. Incubate the µ-Slide for one hour at 37°C.
9. Inject culture medium to fill the channel completely and to eliminate air bubbles formed in the interconnections. For that, slowly perfuse through the channels with ca. 40 µl. Incline the µ-Slide to facilitate the removal of trapped air bubbles. Keep the outlet higher than the filling inlet. This way, floating air bubbles can be pushed out easier. Repeat this step if necessary.



10. Fill each Luer reservoir with 60 µl cell-free medium.



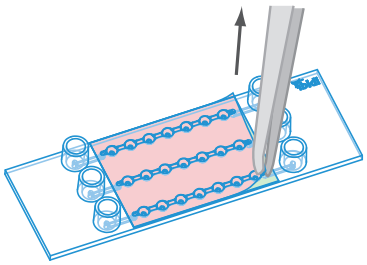
Note:

Cell aggregates, like spheroids and organoids, need a certain mechanical stability with tight cell-cell contacts before they can be transferred without possible damage.

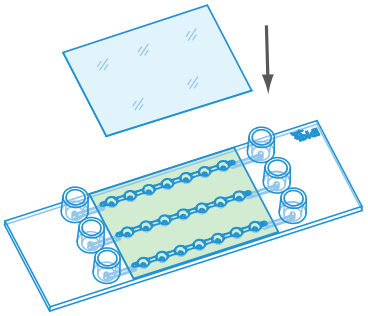
Spheroid Self-organization with Single Cells

The µ-Slide Spheroid Perfusion can be used to generate spheroids directly in the single wells by seeding cells in suspension.

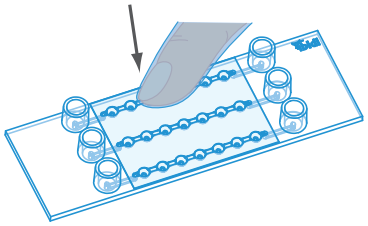
1. Remove the protective foil from the upper side of the µ-Slide. Tweezers are very convenient for this step.



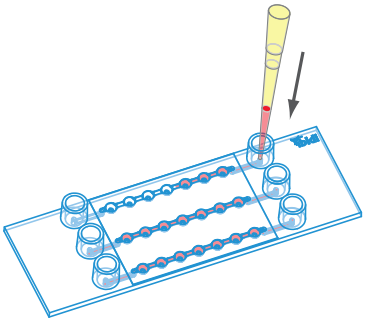
2. Remove the protective foil from the polymer coverslip and place the coverslip on the sticky upper side of the µ-Slide.



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

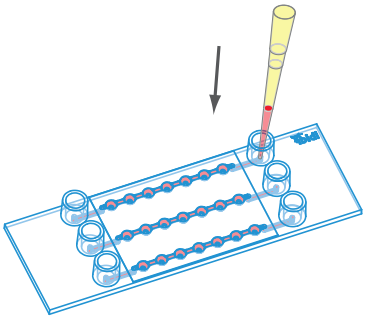


4. Inject 60 µl cell-free culture medium into each channel. Make sure the channels are completely filled. Do not mind incomplete filling or air bubbles at this point.



5. Incubate for 2 hours at 37°C.

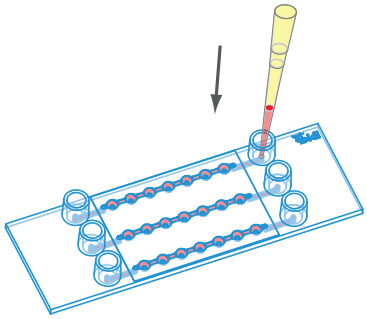
6. Inject culture medium to eliminate air bubbles from the wells and the interconnections. For that, slowly perfuse through the channels with ca. 40 µl. Incline the µ-Slide to facilitate the removal of trapped air bubbles. Keep the outlet higher than the filling inlet. This way, floating air bubbles can be pushed out easier. Repeat this step if necessary.



7. Remove leftover culture medium from the Luer reservoirs.

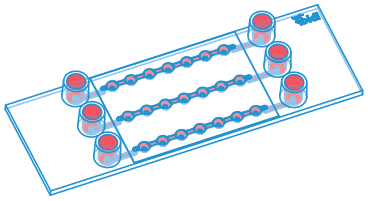
8. Prepare your cell suspension for cell seeding. We recommend using a concentration of 2-10 × 10^5 cells/ml.

9. Inject 45 µl cell suspension directly into each channel. Remove the leftover cell suspensions from the Luer reservoirs. Repeat the seeding step one time for a maximum of well-to-well cell homogeneity.



10. Incubate for one hour at 37°C.

11. Fill each Luer reservoir with 60 µl cell-free medium.



12. Incubate overnight at 37°C.

Tip:

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

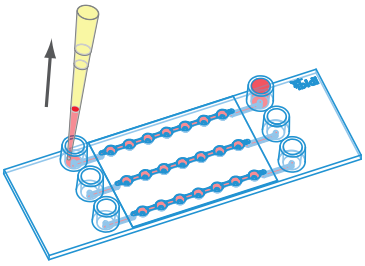
Medium Exchange

Follow the protocol below for an easy medium exchange in the wells/channels. Take care, that the channel never falls dry during the exchange process. This helps you avoiding air bubbles.

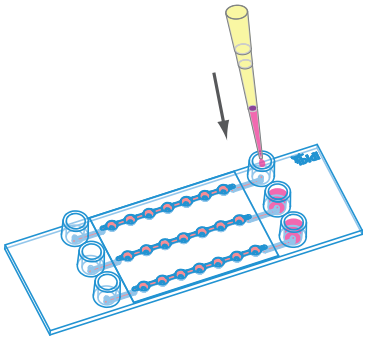
1. If the channel is connected to tubing, pinch off the tubing on both sides of the channel using a tube clamp.

2. Carefully disconnect the tubing.

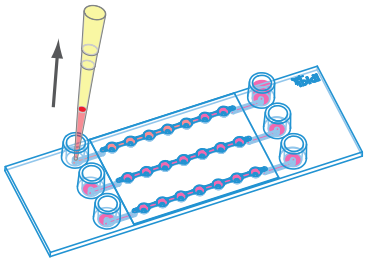
3. Remove all liquid from the Luer ports with a pipette. Take care not to remove the liquid from the channel by pipetting away from the channel inlet.



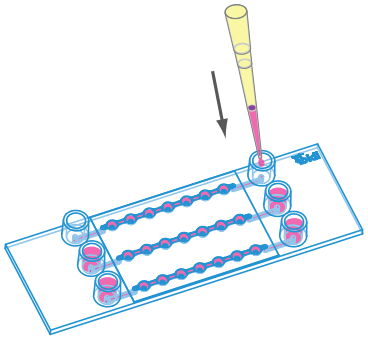
4. Apply 80 µl new solution into one Luer port.



5. Slowly remove 80 µl from the opposite Luer port. If necessary, point the pipet tip inside the channel's inlet making a connection to the liquid inside the channel.



6. Fill each Luer port with 60 µl cell-free medium. For flow connection, fill the ports until they are completely full and meniscus-free (ca. 80 µl).



7. Continue with your experiment.

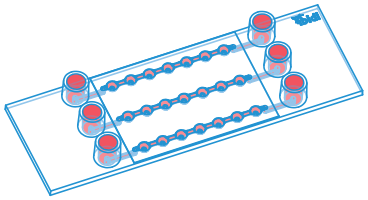
Important!

Take care, that the channel never falls dry during the exchange process. This helps you avoiding air bubbles.

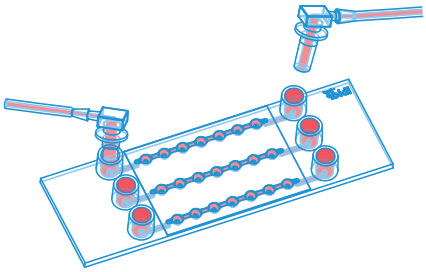
Connecting Tubing for Perfusion

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

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 1) filling the tubing completely and 2) 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 access culture medium with tissue.



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

For a serial connection of channels, please refer to our Application Note 25: Serial Connection Protocol for Luer Slides.

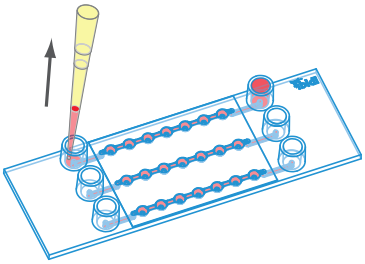
Flow Rates and Flow Setups

For the perfusion of samples in the wells, we either recommend a continuous perfusion or a non-continuous media exchange.
We recommend flow rates between 0.1 and 1.0 ml/min.
Please note that the flow velocity is high in the upper well while the niche is protected from the flow. Therefore, there is no significant shear stress in the niche area.

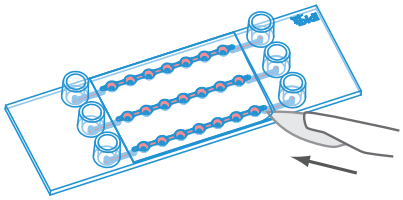
Retrieval of Spheroids

After the experiment, the sample can be retrieved by following the protocol below.

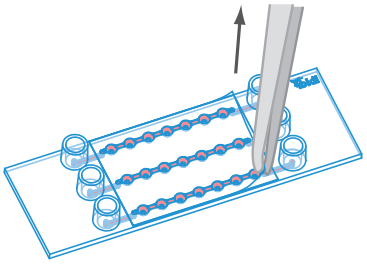
1. Remove all liquid from the Luer ports with a pipette. Take care not to remove the liquid from the channel by pipetting away from the channel inlet.



2. Beginning from one edge, lift the top coverslip. A scalpel or blade facilitates the removal.



3. Grab the unsealed edge of the top coverslip and remove it. Use strong tweezers.



4. Retrieve your sample.

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 a mounting medium (50001) 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 Spheroid 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 7.

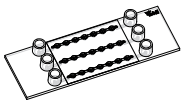
Immersion Oil

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 Spheroid Perfusion is available in three product versions.



Cat. No.	Description
80350	**µ-Slide Spheroid Perfusion Bioinert**: #1.5 polymer coverslip, surface passivation with Bioinert, sterilized
80351	**µ-Slide Spheroid Perfusion Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
80356	**µ-Slide Spheroid Perfusion ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized

For research use only!

Further information 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.