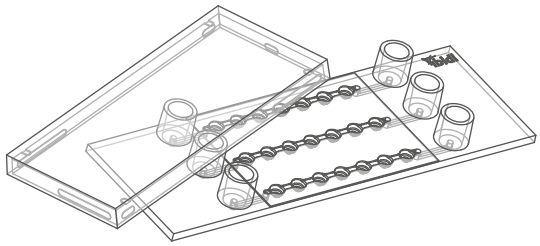
µ-Slide Spheroid Perfusion

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 Spheroid Perfusion is a dedicated flow chamber for 3D cell aggregates. Its unique well geometry ensures continuous nutrient and gas supply for spheroids, organoids or tissue throughout the experiment. Combined with perfusion, it supports maximum cell viability and proliferation while minimizing mechanical shear stress.

This document applies to the following products:

80350	**µ-Slide Spheroid Perfusion Bioinert**
80351	**µ-Slide Spheroid Perfusion Untreated**
80356	**µ-Slide Spheroid Perfusion ibiTreat**

Material

The µ-Slide Spheroid 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.

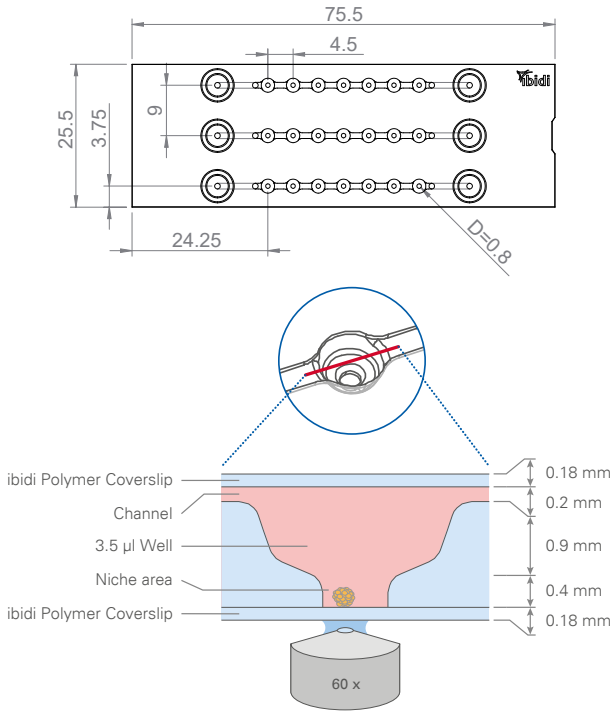
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25 °C), dry place (relative humidity <50%)

Shelf Life	
ibiTreat, Untreated	36 months
Bioinert	36 months

Geometry

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

Specifications	
Outer dimensions	25.5 × 75.5 mm^2
Number of channels	3
Number of wells	3 × 7
Adapters	Female Luer
Volume per well	3.5 µl
Channel volume (total)	45 µl
Volume per reservoir	60 µl
Well height (bottom niche)	0.4 mm
Well height (total)	1.3 mm
Well diameter (bottom)	0.8 mm
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
Top cover	ibidi Polymer Coverslip
Bottom	ibidi Polymer Coverslip



Surface

The µ-Slide Spheroid Perfusion is available with an ibiTreat, Untreated, or Bioinert 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.

The Bioinert surface is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip. It allows no adsorption, coating, or binding of proteins, antibodies, enzymes, and other biomolecules. Therefore, the Bioinert technology provides a stable passivation in cell-based assays for several days or even weeks. The hydrophilic Bioinert surface hinders any protein attachment, thus inhibiting subsequent cell attachment. The Bioinert surface is not biodegradable by cells allowing long-term assays with suspension cells and cell aggregates, such as spheroids, organoids, and embryoid bodies.

TIP – The day before seeding the cells, we recommend placing the cell medium, the slide, and the tubing 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 slide is made of a gas-permeable material, so unpacking is not required for the gas equilibration step.

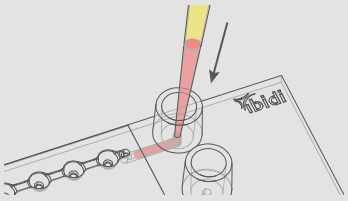
Coating

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

In short, specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 9.7 mm^2 and a volume of 3.5 µl per well.
2. Apply 3.5 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.

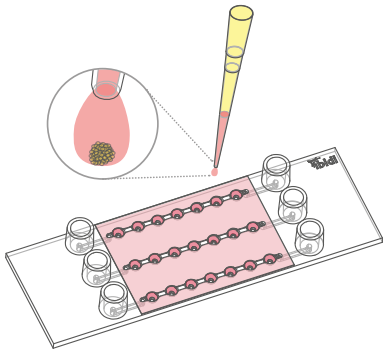
TIP – To completely fill the channel, position the tip directly at the small channel's inlet and gently inject the liquid straight into the channel.



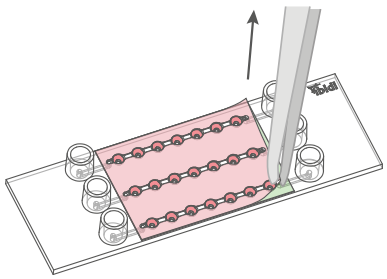
Transfer of Spheroids

Cell aggregates, such as spheroids or organoids, can be transferred into the individual wells of the µ-Slide Spheroid Perfusion using the following protocol. For this application, the use of the Bioinert surface is recommended.

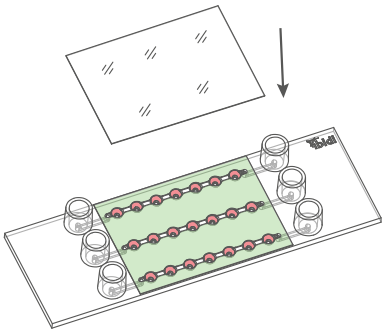
1. Prepare spheroids using your preferred method. Cell aggregates, such as spheroids and organoids, require sufficient mechanical stability and tight cell-cell contacts to withstand transfer without damage. Ensure that the aggregates are well-formed and stable prior to transfer.
2. Transfer one spheroid in a medium droplet into each well. Use a 20 µl pipet tip with an opening wide enough to allow contact-free passage of the spheroid. If necessary, carefully widen the tip opening by cutting it with a sterile scalpel to prevent mechanical damage to the spheroid. Ensure that each well is not overfilled with medium.



3. Check the integrity of the spheroids under a microscope to ensure they remain intact and undamaged after transfer.
4. Remove the protective foil from the upper side of the slide.

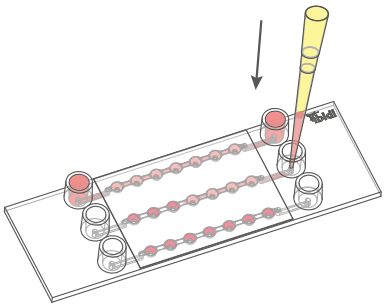


5. Remove the protective foil from the enclosed polymer coverslip and place it onto the adhesive part of the slide, ensuring a tight seal between them. Press down with your fingers to strengthen the connection.

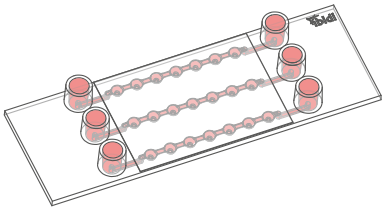


6. Incubate the slide for one hour at 37 °C and 5% CO_2.

7. Slowly perfuse approximately 40 µl of culture medium through the channels to ensure complete filling. Place the pipet tip directly onto the small inlet of the channel. Tilt the slide to help release trapped air bubbles, keeping the outlet higher than the filling inlet. Repeat this step if necessary.



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



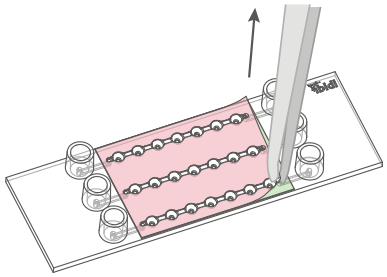
9. Continue with your experiment.

NOTE – For the perfusion of samples in the wells, we recommend using either continuous perfusion or non-continuous media exchange.

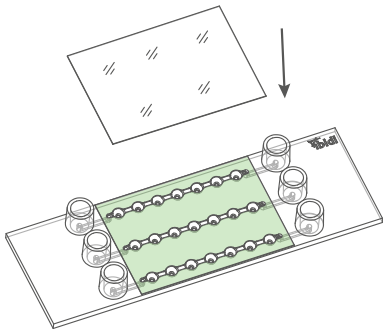
Spheroid Self-Organization with Single Cells

The µ-Slide Spheroid Perfusion enables the generation of spheroids directly within individual wells. For this application, the use of the Bioinert surface is recommended. To do this, follow the protocol below.

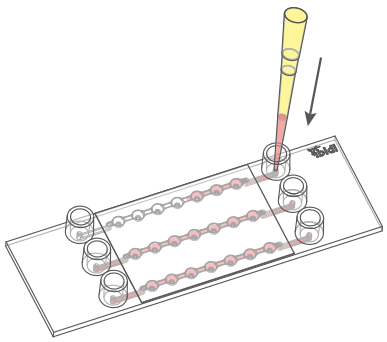
1. Remove the protective foil from the upper side of the slide.



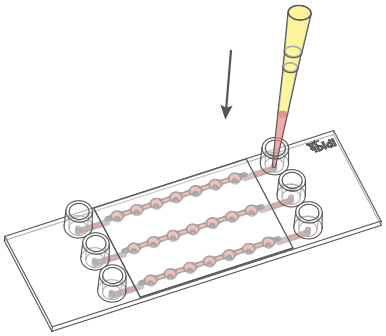
2. Remove the protective foil from the enclosed polymer coverslip and place it onto the adhesive part of the slide, ensuring a tight seal between them. Press down with your fingers to strengthen the connection.



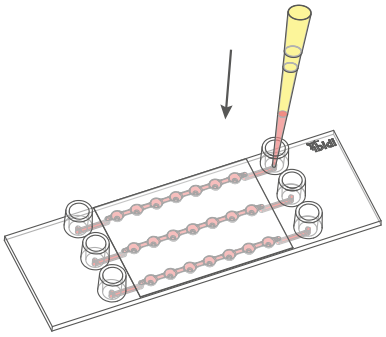
3. Inject 60 µl of cell-free culture medium into each channel. At this stage, incomplete filling or air bubbles can be ignored.



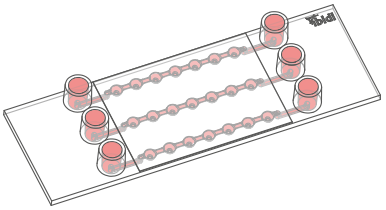
4. Incubate the slide for two hours at 37 °C and 5% CO_2.
5. Slowly perfuse approximately 40 µl of culture medium through the channels to remove air bubbles from the wells and interconnections. Place the pipet tip directly onto the small inlet of the channel. Tilt the slide to help release trapped air bubbles, keeping the outlet higher than the filling inlet. Repeat this step if necessary.



6. Remove any remaining culture medium from the Luer reservoirs.
7. Prepare your cell suspension for seeding. We recommend using a concentration of 2–10 × 10^5 cells/ml.
8. Inject 45 µl of cell suspension directly into each channel. Remove any remaining cell suspension from the Luer reservoirs. Repeat this step once more to ensure optimal cell distribution and maximize well-to-well homogeneity.



9. Incubate the slide for one hour at 37 °C and 5% CO_2.
10. Fill each Luer reservoir with 60 µl cell-free medium.



11. Close the lid. Incubate overnight at 37 °C and 5% CO_2.
12. Continue with your experiment.

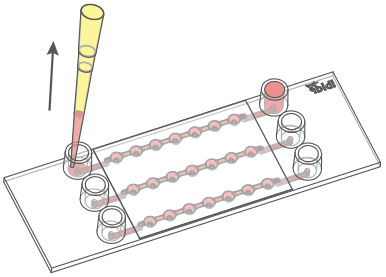
NOTE – For the perfusion of samples in the wells, we recommend using either continuous perfusion or non-continuous media exchange.

Medium Exchange

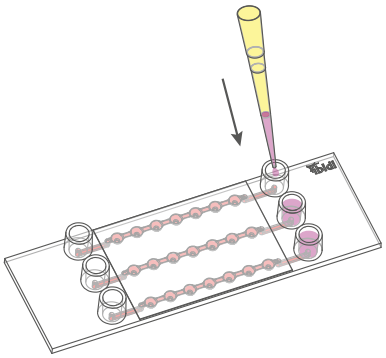
Follow the protocol below for easy medium exchange in the wells and channels.

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

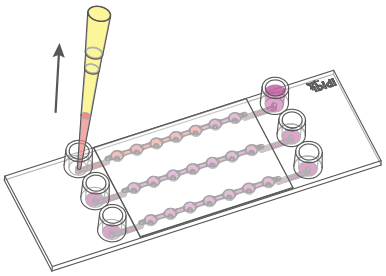
1. If the µ-Slide Spheroid Perfusion is connected to a pump, disconnect the tubing from the channel first.
2. Remove the medium from the Luer ports with a pipet, putting the pipet tip away from the channel inlet. Do not remove any liquid from the channel itself.



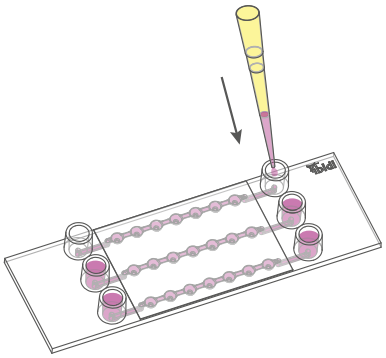
3. Fill 80 µl of fresh medium into one of the Luer ports, which will replace the channel volume by gravity flow.



4. 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.



5. Repeat the medium exchange if necessary.
6. Refill each Luer port with 60 µl cell-free medium. Before reconnecting the tubing, ensure that the ports are completely filled without forming a meniscus (approximately 80 µl total volume).

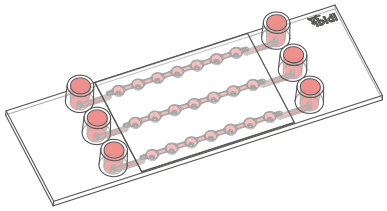


7. Continue with your experiment.

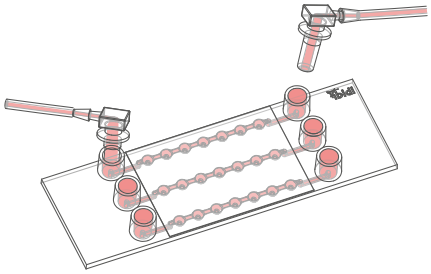
Connecting Tubing for Perfusion

The µ-Slide Spheroid Perfusion is compatible with the ibidi Pump System as well as other pump setups designed for cell cultivation under flow conditions. For use under flow, please follow the protocol below after cell attachment, spheroid formation, or transfer of pre-formed spheroids.

1. Fill both Luer ports of the designated flow channel completely with cell-free medium to ensure an air bubble-free connection of the tubing.



2. Prepare the perfusion system: Fill the tubing completely with medium, then pinch it off using a screw clamp or hose clip.
3. Connect the male Luer ends of the clamped tubing to the Luer ports one at a time, ensuring no air is trapped. Remove any excess medium with a tissue.



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

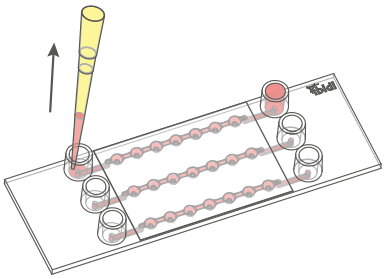
TIP – We recommend flow rates between 0.1 and 1.0 ml/min.
Please note that the flow velocity is high in the upper well while the niche is protected from the flow. Therefore, there is no significant shear stress in the niche area.

For a serial connection of several slides with each other, please refer to Application Note 25: Serial Connection of Luer-Slides for Flow Experiments.

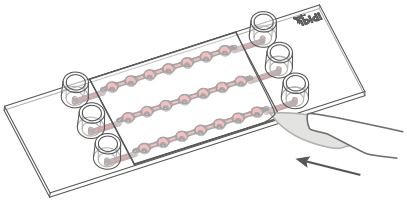
Retrieval of Spheroids

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

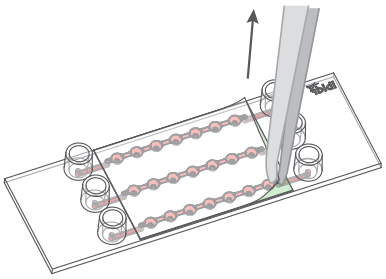
1. Remove all liquid from the Luer ports using a pipet. To avoid disturbing the liquid inside the channel, pipet carefully away from the channel inlet.



2. Starting at one edge, gently lift the top coverslip. Using a scalpel or blade can help facilitate removal.



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



4. Retrieve your sample.

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

Chemical Compatibility

The following table provides 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, 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

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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.