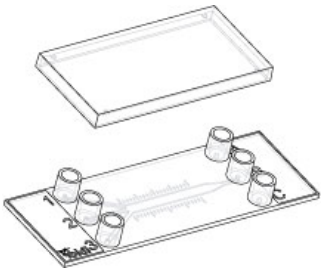


The ibidi product family is comprised of a variety of µ-Slides and µ-Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ-Slide III 3in1 is designed for flow assays with different liquids merging into one channel. It can be connected to a pump and enables you to observe cells under switchable flow conditions. The design allows generating fluid stable concentration profiles in the main channel for e.g. chemotaxis experiments. The microfluidic system can generate spatially and temporally controlled gradients of chemotacic factors by laminar flow.

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.

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.

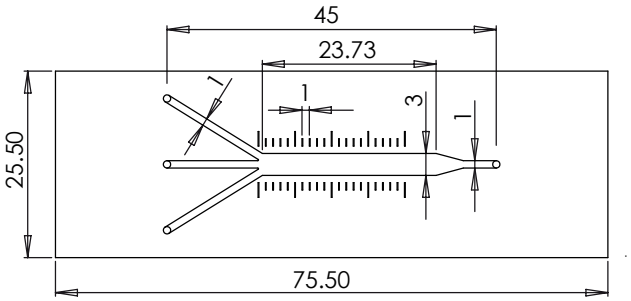
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15–25°C)

Shelf Life	
ibiTreat, Uncoated	36 months

Geometry

The µ-Slide III 3in1 provides standard slide format according to ISO 8037/1.

Geometry of the µ-Slide III 3in1	
Outer dimensions	25.5 mm x 75.5 mm
Adapters	Female Luer
Volume per reservoir	60 µl
Total channel volume	60 µl
Height of all channels	0.4 mm
Width of channels thin/thick	1/3 mm
Total growth area	1.23 cm^2
Coating area using 60 µl	3.05 cm^2
Distance of scale bars	1 mm
Bottom	No. 1.5 ibidi Polymer Coverslip



Surface

The tissue culture-treated ibiTreat surface is a physical surface modification and optimized for adhesion of most cell types. The uncoated surface 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 uncoated and ibiTreat surfaces, since some proteins and

biomolecules adhere differently to hydrophobic or hydrophilic polymer surfaces.

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:

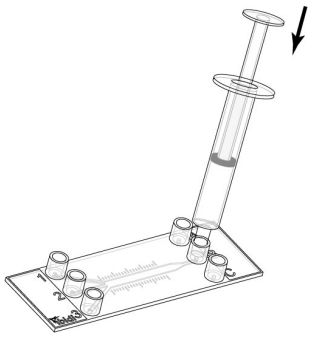
1. Prepare your coating solution according to the manufacturer's specifications or reference.
2. Apply 60 µl to adapter B (handling see below) and leave at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. The µ-Slide III 3in1 is ready to be used. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Tip:

For washing you can add the buffer into one channel end and simultaneously aspirate it on the other side. Take care that all of the three channels are washed.

Filling and Handling

- Always fill the channel from adapter B as shown in the picture.
- When using a pipet make sure you place the tip directly onto the small channels inlet.
- Especially the uncoated, hydrophobic channel can be filled much easier by using a small volume syringe with a Luer tip.
- Make sure that all adapters are completely filled before Luer connectors are plugged in. Otherwise air bubbles will be trapped.



Seeding Cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 3–7 × 10^5 cells/ml results in a 20% optical confluent cell layer after attachment.
- A seeding density of 1–4 × 10^6 cells/ml creates a 100% optical confluent cell layer after cell attachment.
- Apply 60 µl cell suspension into adapter B of the µ-Slide. Quick dispensing helps to avoid trapped air bubbles.
- Cover reservoirs with the supplied lid. Incubate at 37°C and 5 % CO_2 as usual.
- Await cell attachment in order not to flush out the cells. Afterwards fill each reservoir with 60 µl cell free medium.
- Connect the µ-Slide to the pump and conduct your perfusion experiment.

Important!

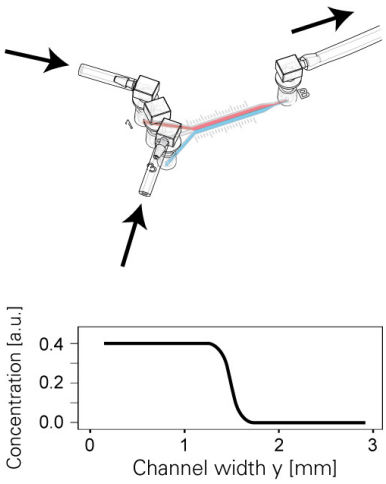
The day before seeding the cells we recommend placing the cell medium and the µ-Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Exchanging Medium

Aspirate all four reservoirs and fill 60 µl of fresh medium into reservoir B, which will replace the channel volume by gravity flow. Repeat this step three times until each reservoir is filled with 60 µl.

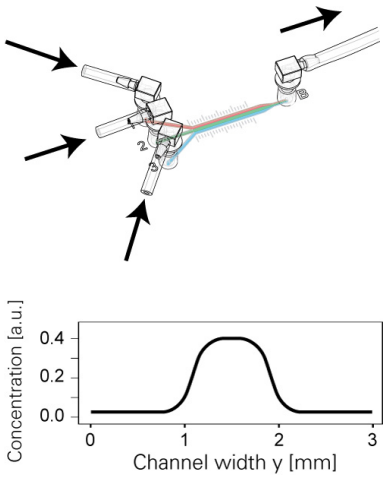
Fluid Connections and Gradient Shapes

2 in 1

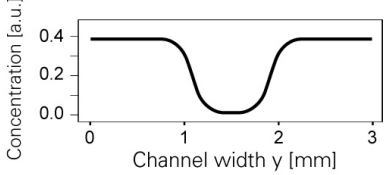


Cliff-shaped Gradients

3 in 1



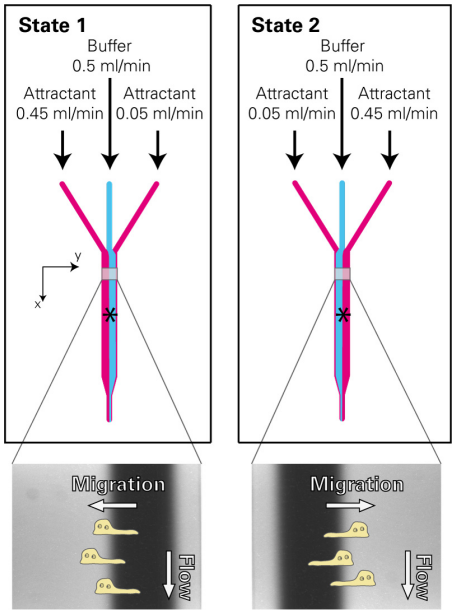
Hill-shaped Gradients



Cup-shaped Gradients

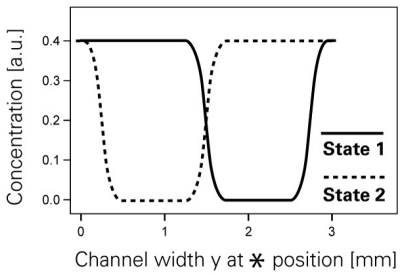
Example Experiment – Cells in Temporally Controlled Gradients

The following example experiment illustrates the idea how to setup a switchable chemical gradient in the large channel for a chemotaxis experiment.

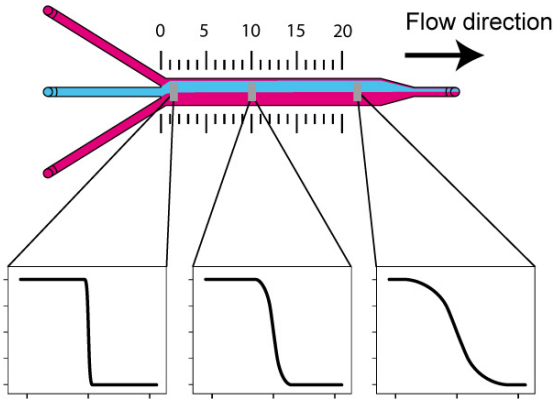


Concentration profiles can be visualized by using a fluorescent dye e.g. rhodamine. Switching time between state 1 and state 2 can range from seconds to hours, depending on the speed of the cells' response.

The concentration profile created is always sigmoid shaped and is depending on overall flow rate and position inside the channel.



All flow rates should be adjusted in a way that the point of inflection of the sigmoid is at identical position in state 1 and state 2.



The longer liquids are in contact next to each other the smoother the sigmoid shape.

Since flow is used to keep the gradients stable, there is always a shear stress applied to the cells. Please perform control experiments with the experimental flow rate to exclude polarization effects from the flow itself. Flow rates and corresponding shear stress can be found in Application Note 11: Shear Stress and Shear Rates.

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 III 3in1. 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	yes, without lid
Mineral oil	no
Silicone oil	yes
Immersion oil	See **Immersion Oil** on page 4.

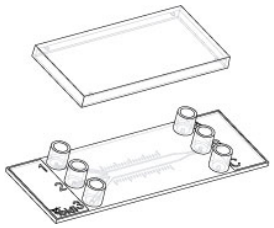
Immersion Oil

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

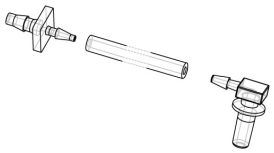
Ordering Information

The µ-Slide III 3in1 is available with different surfaces. See table below for choosing your µ-Slide III 3in1.



Cat. No.	Description
80316	**µ-Slide III 3in1 ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
80311	**µ-Slide III 3in1 Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized

Tube Adapter Set



Cat. No.	Description
10831	**Tube Adapter Set**: 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.