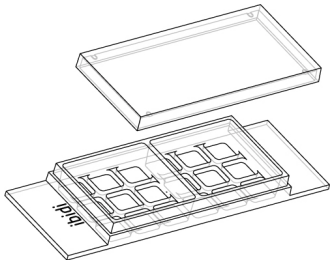

The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength. The µ–Slide 2 Well Co–Culture harbours two arrays of 3×3 square fields where cells can be cultivated and investigated with microscopical methods. It is intended for checking out experimental parameters like antibody dilution, seeding density or most effective drug concentrations. A special application is the co-cultivation of different cell types which share one medium supernatant but grow separately from each other.

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 3.

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 2 Well Co–Culture provides a standard slide format according to ISO 8037/1.

Geometry of the µ–Slide 2 Well Co–Culture	
Outer dimensions in mm (w × l)	25.5×75.5
Number of major wells	2
Dimensions of major wells in mm (w × l × h)	21.5 × 23.6 × 6.8
Volume per major well	600 µl
Number of minor wells	2 × 9
Dimensions of minor wells in mm (w × l × h)	6.1 × 6.8 × 1.3
Volume per minor well	70 µl
Growth area per minor well	0.4 cm^2
Coating area per minor well	0.55 cm^2
Total height with lid	8 mm
Bottom	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

Specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications or reference.
2. Apply 70 µl per inner well and leave at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. 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

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 5–10 × 10^4 cells/ml suspension should result in a confluent layer within 2–3 days.
- Use the center minor well for recipient cells and the 8 outer wells for feeder cells.
- Apply 40-60 µl cell suspension into each minor well of the µ–Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover reservoirs with the supplied lid. Incubate at 37 °C and 5 % CO_2 as usual.
- After cell attachement fill 400-600 µl into each large reservoir, allowing the cells to share factors.

Undemanding cells can be left in their seeding medium for up to three days and grow to confluency there. However, best results might be achieved when the medium is changed every 1–2 days. Carefully aspirate the old medium and replace by 1.2 ml fresh medium.

Please also see our Application Note 10, "Co-Cultivation Using ibidi µ–Slides".

Cell Microscopy and Solvents for Fixation

To analyze your cells, no special preparations are necessary. Cells can be observed live, or fixed directly in the µ–Slide 2 Well Co–Culture, preferably on an inverted microscope. Due to the thin bottom, high resolution microscopy is possible. The material is compatible to most fixatives, like acidic acid, alcohols and PFA. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on www.ibidi.com. For optimal results in fluorescence microscopy and storage of stained probes ibidi provides a mounting medium (50001) optimized for µ-Dishes and µ-Slides.

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
Zeiss	Immersol 518 F	444960	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012
Leica	Immersion Liquid	11513859	n.a.	03/2011
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017

Selected References

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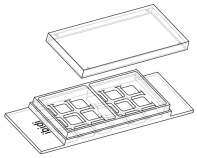
C. Hagen, P. Guttmann, B. Klupp, S. Werner, S. Rehbein, T. C. Mettenleiter, G. Schneider, and K. Günewald. Correlative VIS-fluorescence and soft X-ray cryo-microscopy/tomography of adherent cells. *Journal of Structural Biology*, 2012. doi: 10.1016/j.jsb.2011.12.012.

M. Köttgen, A. Hofherr, W. Li, K. Chu, S. Cook, C. Montell, and T. Watnick. Drosophila Sperm Swim Backwards in the Female Reproductive Tract and Are Activated via TRPP2 Ion Channels. *PLoS ONE*, 2011. doi: 10.1371/journal.pone.0020031.

R. J. Scheubel, J. Holtz, I. Friedrich, J. Borgermann, S. Kahrstedt, A. Navarrete-Santos, R. E. Silber, and A. Simm. Paracrine effects of CD34 progenitor cells on angiogenic endothelial sprouting. *International Journal of Cardiology*, 2008. doi: 10.1016/j.ijcard.2008.10.009.

Ordering Information

The µ–Slide 2 Well Co–Culture is available with different surfaces. See table below for choosing your µ–Slide 2 Well Co–Culture.



Cat. No.	Description
81806	**µ–Slide 2 Well Co–Culture ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
81801	**µ–Slide 2 Well Co–Culture Uncoated**: #1.5 polymer coverslip, hydrophobic, 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.