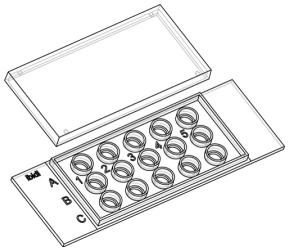


The ibidi product family comprises a variety of different shapes of µ–Slides and µ–Dishes which all have been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ–Slide Angiogenesis is a cell culture product for angiogenesis assays and direct cell culture. Cells can be grown on gel matrices, e.g. collagen gels, hyaluronic gels or BD Matrigel™ (Becton–Dickinson) or directly on the ibidi coverslip-like plastic bottom.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates consist of a plastic with highest optical quality. The bottom material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. 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 temperature stable up to 80°C/175°F only. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the plastic bottom which should not be covered.

Optical Properties ibidi Standard Bottom	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	microscopy plastic

µ–Slide Surfaces

The µ–Slide Angiogenesis is available with ibiTreat and the uncoated surface. The ibiTreat surface is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested for good cell growth. Uncoated is a very hydrophobic surface and allows no direct cell growth. If, for any reason, you might need special coatings such as Collagen IV, Fibronectin, Poly-L-Lysine, and Poly–D–Lysine these can be provided on request. In this case only high quality substrates are used [1].

A detailed protocol for applying gel matrices is provided in the section Coating your µ–Slide Angiogenesis.

Geometry of the µ-Slide Angiogenesis

The µ-Slide Angiogenesis provides standard slide format according to ISO 8037/1. The well to well distance of 9 mm (like 96 well plates) allows using multichannel pipettes.

Geometry of the µ-Slide Angiogenesis	
Number of wells	15
Volume inner well	10 µl
Diameter inner well	4 mm
Depth inner well	0.8 mm
Volume upper well	50 µl
Diameter upper well	5 mm
Growth area inner well	0.125 cm^2
Coating area using 10 µl	0.23 cm^2
Bottom matches coverslip	No. 1.5

Coating your µ–Slide Angiogenesis

The uncoated µ–Slide must be coated to promote cell adhesion. If you like to establish a certain coating for your demands we recommend testing your coating procedure on uncoated and ibiTreat µ–Slides, since we have observed that some biomolecules adhere differently to hydrophobic or hydrophilic plastic surfaces.

- Prepare your gel matrix according to the manufacturer's protocol or reference.
- Fill the inner well with 10 µl liquid gel. Avoid air bubbles.
- Let the gel polymerize under appropriate conditions.
- Use as soon as possible.
- If storage is needed fill sterile water around the wells to generate a humidified environment to hinder evaporation.

Non-gel based coatings are also possible. Please use 10 µl coating solution and calculate with an area to be coated of 0.23 cm^2 per well. Refer to our Application Note 08 'Cell culture coating' on www.ibidi.com.

[1] Collagen IV, BD Cat.-Nr. 35 6233, Fibronectin, BD Cat.–Nr. 354008, Poly–L–Lysin, Sigma Cat.–Nr. P4832, Poly–D–Lysin, BD Cat.–Nr. 354210

Seeding Cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, we recommend 1–3 $\times 10^5$ cells/ml.
- Apply 50 µl of the cell suspension into the upper well. Do not touch the gel matrix with the pipet tip.
- Incubate at 37°C and 5% CO_2 as usual.
- Conduct your experiment.

Depending on the cell type medium exchange is necessary every 1–2 days. Carefully aspirate the old medium and replace it by 50 µl fresh medium.

Optionally, µ–Slide Angiogenesis can be used for the following assays:

- Fill the inner well with a gel matrix and culture pieces of tissue or cell spheroids on it.
- Fill the inner well with cells suspended inside a gel matrix.
- Culture cells without a gel matrix directly in the minor wells. Use approx. 3 $\times 10^5$ cells/ml and a volume of 10 µl. After cell attachment, add 50 µl cell–free medium to fill the upper well. Please keep in mind that the uncoated version does not provide direct cell growth. Use the tissue culture treated version (ibiTreat) or your specific coating instead.

Tip:

Air bubbles in the gel can be reduced by equilibrating the slide before usage inside the incubator overnight. For less evaporation the space in–between the wells can be filled with sterile water.
In case bent gel surfaces are created, increase or decrease the amount of gel used, until you get flat and even gels.

Preparation for Cell Microscopy

When gel matrices are used the optical quality and the use of high magnification objective lenses might be restricted. Without any gel cells can be observed live or fixed directly in the wells on an inverted microscope. You can use any fixative of your choice. The plastic 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, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of 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

µ–Slide Angiogenesis Family

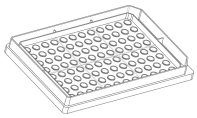
The µ–Slide Angiogenesis family is available with different surfaces and formats. See table below for choosing your µ–Slide and µ–Plate Angiogenesis, respectively.

µ–Slide Angiogenesis

Ordering number	Treatment or Coating	Characteristics
81506	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
81501	uncoated, sterile	hydrophobic
81531	PEN-membrane, 1 µm, sterile*	for Laser Microdissection

* The PEN foil does not fit to standard cover slip thickness.

µ–Plate Angiogenesis 96 well



Ordering number	Treatment or Coating	Characteristics
89646	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated, round wells

References

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E. Vo, D. Hanjaya-Putra, Y. Zha, S. Kusuma, and S. Gerecht. Smooth-muscle-like cells derived from human embryonic stem cells support and augment cord-like structures in vitro. *Stem Cell Reviews and Reports*, 2010. doi: 10.1007/s12015-010-9144-3.

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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 46 17 0. All products are developed and produced in Germany.