

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-Plate Angiogenesis 96 well allows you to perform tube formation assays on gel, combined with high resolution microscopy in a standard multi-well format.

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 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

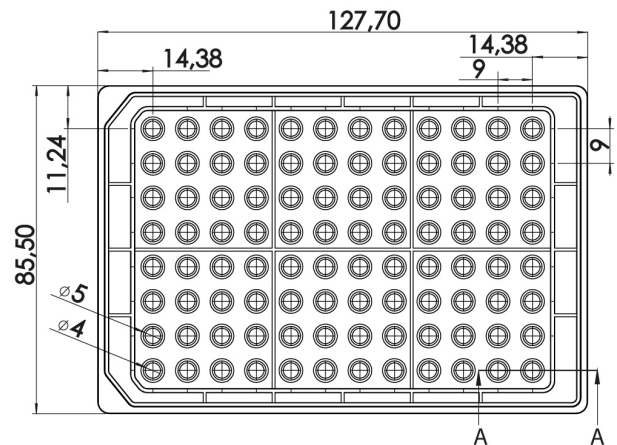
Shipping conditions	Ambient
Storage conditions	RT (15-25°C)

Shelf Life of Different Surfaces

ibiTreat, Glass Bottom, ESS	36 months
Collagen, Poly-L-Lysine	18 months

Geometry

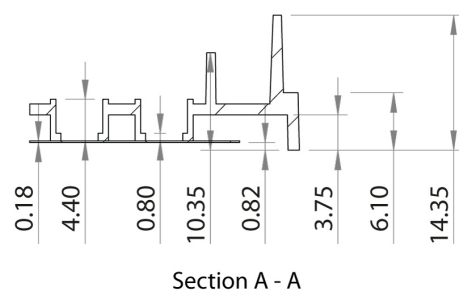
The μ-Plate Angiogenesis 96 Well provides standard geometry and numbering (A-H, 1-12). The bottom of the μ-Plate Angiogenesis 96 Well provides a high accuracy.



The μ-Plate Angiogenesis 96 Well meets all important values of the ANSI/SBS Standards (1-2004, 2-2004, 3-2004 and 4-2004).

Dimensions of the μ-Plate Angiogenesis 96 Well in mm

Length	127.7	± 0.2
Width	85.5	± 0.2
Height with lid	16.5	± 0.4
Height without lid	14.4	± 0.4
Well to well distance	9.0	± 0.1



Single Well Parameters	
Volume inner well	10 μl
Diameter inner well	4 mm
Depth inner well	0.8 mm
Volume upper well	70 μl
Diameter upper well	5 mm
Growth area inner well	0.125 cm ²
Coating area using 10 μl	0.23 cm ²

Accuracy of the Bottom	
Inner well flatness	± 5 μm
Whole plate flatness	± 25 μm
Bottom matches coverslip	No. 1.5

Surface

The μ-Plate Angiogenesis 96 Well is available with ibiTreat 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.

A specific coating of the μ-Plate Angiogenesis 96 Well can be done yourself following the procedure in section "Coating".

Coating your μ-Plate Angiogenesis 96 Well

In tube formation assays the μ-Plate Angiogenesis 96 Well is coated with a 0.8 mm thick layer of gel matrix.

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

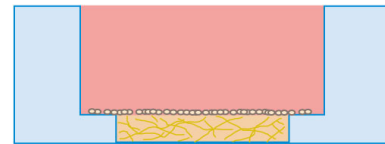
Non-gel based coatings are also possible. Please use 10 μl coating solution and calculate with an area to be coated of 0.23 cm² per well. Further information about coatings is provided in [Application Note 08 "Cell culture coating"](#).

Remove the Protection Film before Usage

The bottom of the μ-Plate Angiogenesis 96 Well is covered with a film to protect the optical quality of the plastic surface. Please pull off the protection film before usage!

Tube Formation Assays

In a tube formation assay cells are seeded on top of the polymerized gel matrix:



1. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, we recommend 1–3 × 10⁵ cells/ml.
2. Apply 70 μl of the cell suspension into the upper well. Do not touch the gel matrix with the pipet tip.
3. Cover the μ-Plate Angiogenesis 96 Well with the supplied lid. Incubate at 37°C and 5 % CO₂ as usual.
4. Conduct your experiment.
5. Depending on the cell type, medium exchange is necessary every 1–2 days. Carefully aspirate the old medium and replace it by 70 μl fresh medium.

For a detailed protocol please refer to [Application Note 19 "Tube Formation"](#) and [Application Note 5 "Tube Formation in μ-Plate Angiogenesis 96 well"](#).

Further information about the optimization of experimental parameters and data analysis is provided in [Application Note 27 "Tube Formation - Data Analysis"](#).

Tip:

Air bubbles in the gel can be reduced by equilibrating the μ-Plate Angiogenesis 96 Well before usage inside the incubator overnight.

In case bent gel surfaces are created, increase or decrease the amount of gel used, until you get flat and even gels.

Tip:

For less evaporation the reservoirs at the edges can be filled with sterile water or agarose. Add agarose to water or buffer solution (e.g. 0.1 g to 10 ml water). Melt agarose solution using a microwave or boiling water bath and allow the solution to cool to ~50°C.

Tip:

You can stack the μ-Plates Angiogenesis to save space in your incubator. This will not affect cell growth. We recommend making batches with up to 6 plates, due to stability reasons.

Seeding Cells in 2D

You can also use the μ-Plate Angiogenesis 96 Well for a standard 2D cell culture without gel matrix.



1. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a $1.8-4.3 \times 10^5$ cells/ml suspension should result in a confluent layer within 2–3 days.
2. Apply 10 μl cell suspension into each well of the μ-Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells.
3. Cover the slide with the supplied lid. Incubate at 37°C and 5% CO₂ as usual.
4. After cell attachment, add 70 μl cell-free medium to fill the upper well.

Attention!

Avoid evaporation during seeding and cell culture in the incubator! We recommend placing the μ-Plate Angiogenesis 96 Well in an extra humidity chamber (e.g. a Petri Dish with wetted paper).

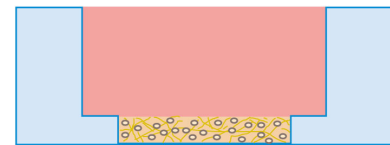
Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. How-

ever, best results might be achieved when the medium is changed every 1–2 days. Carefully aspirate the old medium and replace it by 80 μl fresh medium per well.

Experimental Setups

Alternatively, the μ-Plate Angiogenesis 96 Well can be used for the following assays:

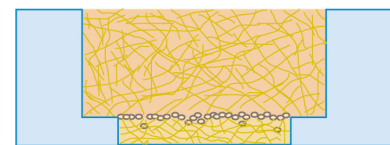
- Fill the inner well with cells suspended inside a gel matrix. After gel polymerization, add 70 μl cell-free medium to fill the upper well.



- Fill the inner well with a gel matrix and culture pieces of tissue or cell spheroids on it.



- Sandwich Cell Culture: Fill the inner well with a gel matrix. Seed cells on top of the polymerized gel and imbed the cells with 70 μl gel in the upper well.



- Fill the inner well with a low volume of the gel matrix, e.g. 8 μl. Seed cells on top of the polymerized gel. If necessary gently shake the slide to make the cells slide into the middle of the well.



- Fill the inner well with fibroblasts suspended inside a gel matrix. Seed cells on top of the polymerized gel. Overlay the cell layer with medium and incubate for invasion of the cells into the gel matrix.



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, use only the immersion oils specified in the table. The use of a non-recommended oil could lead to the damage 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

References

- P. S. Chen, Y. W. Shih, H. C. Huang, and H. W. Cheng. Diosgenin, a Steroidal Saponin, Inhibits Migration and Invasion of Human Prostate Cancer PC-3 Cells by Reducing Matrix Metalloproteinases Expression. *PLoS ONE*, 2011. doi: 10.1371/journal.pone.0020164.
- J. Searle, M. Mockel, S. Gwosc, S. A. Datwyler, F. Qadri, G. I. Albert, F. Holert, A. Isbruch, L. Klug, and D. N. Muller. Heparin Strongly Induces Soluble fms-Like Tyrosine Kinase 1 Release In Vivo and In Vitro. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2011. doi: 10.1161/ATVBAHA111.237784.
- 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.

Ordering Information

The μ-Plate Angiogenesis 96 Well family is available with different surfaces and formats. See table below for choosing your μ-Slide and μ-Plate Angiogenesis, respectively.

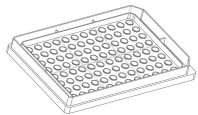
μ-Slide Angiogenesis



Cat. No.	Description
81506	μ-Slide Angiogenesis ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized
81501	μ-Slide Angiogenesis Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized
81531	μ-Slide Angiogenesis Microdissection: PEN-membrane*, sterilized

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

μ-Plate Angiogenesis 96 well



Cat. No.	Description
89646	μ-Plate Angiogenesis 96 well ibiTreat: #1.5 polymer coverslip, tissue culture treated, 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.

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