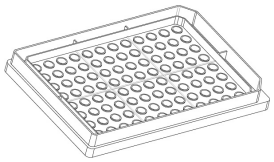


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised 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 bottom material 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 Standard Bottom	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	microscopy plastic/ polymer coverslip

Please note! The ibidi standard bottom is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 3.

Geometry

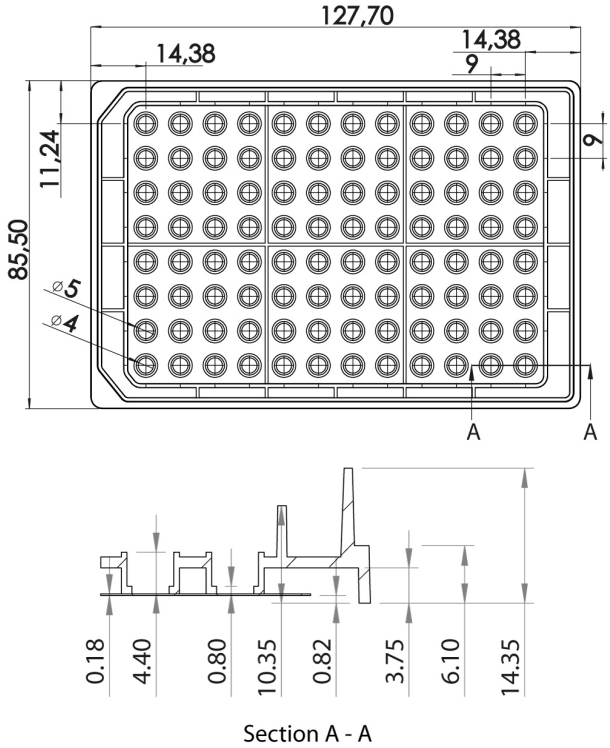
The µ–Plate Angiogenesis 96 well provides standard geometry and numbering (A-H, 1-12). The bottom of the µ–Plate Angiogenesis 96 well provides a high accuracy.

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	ibidi Standard Bottom

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



Section A - A

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-Lysine	18 months
Fibronectin	4 months

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".

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!

Coating

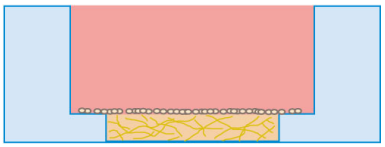
In tube formation assays the µ–Plate Angiogenesis 96 well is coated with a 0.8 mm thick layer of gel matrix.

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 in the reservoirs at the edges of the plate.

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. Further information about coatings is provided in Application Note 08 "Cell culture coating".

Seeding Cells

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 $\times 10^5$ 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 % CO_2 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.

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:

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

Tip:

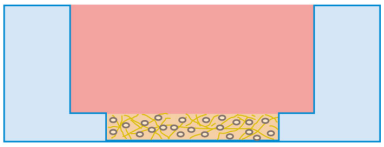
You can stack the µ–Plates Angiogenesis to save space in you incubator. This will not affect cell growth. We recommend making batches with up to 6 plates, due to stability reasons.

Experimental Setups

Alternatively, the µ–Plate Angiogenesis 96 well can be used for the following assays:

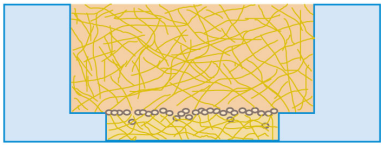
- Culture cells without a gel matrix directly in the minor wells.

- 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
ibidi	Immersion Oil	(ibidi) 50101
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

µ–Plate Angiogenesis 96 well Family

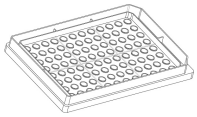
The µ–Plate Angiogenesis 96 well family is available with different surfaces and formats. See table below for choosing your µ–Slide and µ–Plate Angiogenesis, respectively.

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

References

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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: 10.1161ATVBAHA111.237784.

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.

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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 4617 0. All products are developed and produced in Germany.