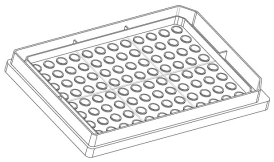


The ibidi product family is comprised of a variety of µ-Slides and µ-Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ-Plate Angiogenesis 96 Well is a cell culture product for tube formation, angiogenesis assays and direct cell culture in a standard multi-well format. Cells can be grown on or in gel matrices, e.g. Matrigel™ or directly on the ibidi Polymer Coverslip.

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

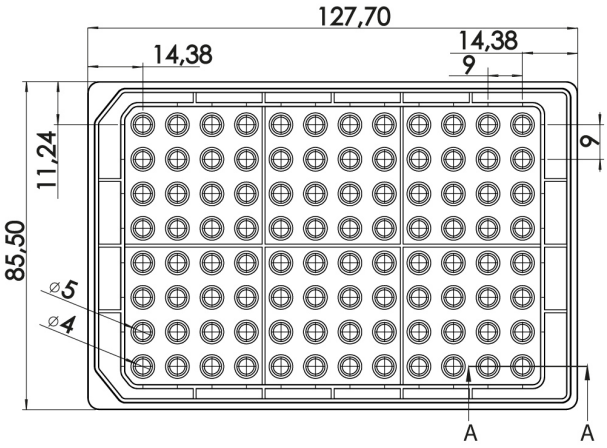
The µ-Slides, µ-Dishes and µ-Plates are sterilized and welded in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is listed in the following table.

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

Shelf Life	
ibiTreat	36 months

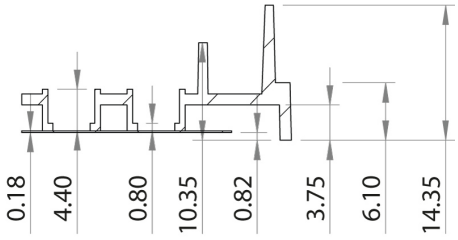
Geometry

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/SLAS (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



Section A - A

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
Well clearance	0.82 mm

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.

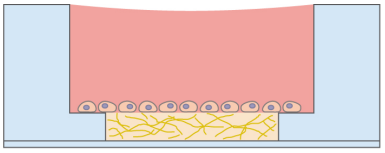
Non-gel based coatings are also possible. Please use 10 µl coating solution and calculate with an area to be coated of 0.23 cm^2 per well. Further information about coatings is provided in Application Note 08 "Cell culture coating".

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 $\times 10^5$ 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.
In case bent gel surfaces are created, increase or decrease the amount of gel used, until you get flat and even gels.

Tip:

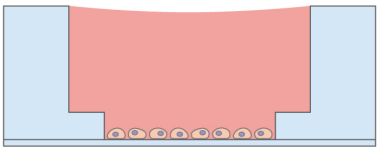
For less evaporation the reservoirs at the edges can be filled with sterile water or agarose. Add agarose to water or buffer solution (e.g. 0.1 g to 10 ml water). Melt agarose solution using a microwave or boiling water bath and allow the solution to cool to ~50°C.

Tip:

You can stack the µ-Plate Angiogenesis 96 Well 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 × 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-Plate Angiogenesis 96 Well. 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 % CO_2 as usual.
4. After cell attachment, add 70 µl cell-free medium to fill the upper well.

Attention!

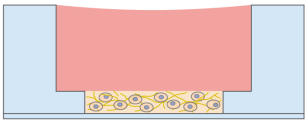
Avoid evaporation during seeding and cell culture in the incubator! We recommend placing the µ-Plate Angiogenesis 96 Well in an extra humidity chamber (e.g. a Petri Dish with wetted paper).

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

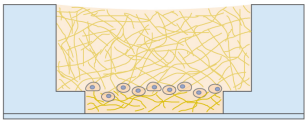
Experimental Setups

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

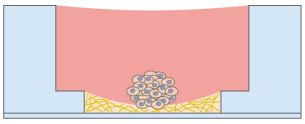
- Fill the inner well with cells suspended inside a gel matrix. After gel polymerization, add 70 µl cell-free medium to fill the upper well.



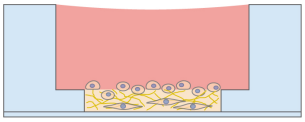
- Sandwich Cell Culture: Fill the inner well with a gel matrix. Seed cells on top of the polymerized gel and imbed the cells with 70 µl gel in the upper well.



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



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.

Note:

When gel matrices are used the optical quality and the use of high magnification objective lenses might be restricted.

Chemical Compatibility

The table below provides some basic information on the chemical and solvent compatibility of the µ-Plate Angiogenesis 96 Well. 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
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

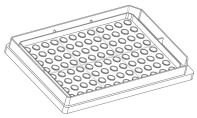
Ordering Information

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

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 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.