

## Instructions

### μ-Slide 15 Well 3D (formerly μ-Slide Angiogenesis)



The ibidi product family is comprised of a variety of μ-Slides, μ-Dishes, and μ-Plates 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 15 Well 3D (formerly μ-Slide Angiogenesis) is a cell culture product for tube formation, angiogenesis assays and direct cell culture. Cells can be grown on or in gel matrices, e.g. Matrigel<sup>™</sup> or directly on the ibidi Polymer Coverslip.

## Overview

This document is applicable to the following product numbers:

Cat. No.	Product Name
81506	μ-Slide 15 Well 3D ibiTreat: #1.5 polymer coverslip, tissue culture treated, sterilized, individually packed
81501	μ-Slide 15 Well 3D Uncoated: #1.5 polymer coverslip, hydrophobic, sterilized, individually packed

## Material

ibidi μ-Slides, μ-Dishes, and μ-Plates are made of a polymer 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 intended for one-time use and are not autoclavable, since they are only temperature-stable up to 80°C/175°F. Please note that gas exchange between the medium and the 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, Uncoated	36 months

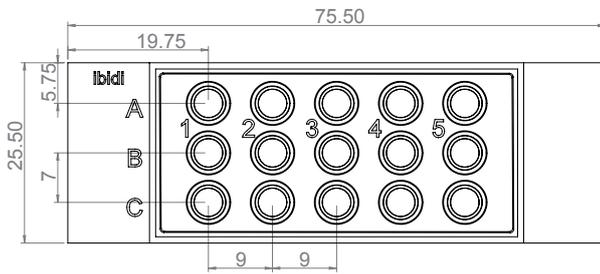
## Geometry

The μ-Slide 15 Well 3D 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 15 Well 3D	
Outer dimensions (w x l)	25.5 x 75.5 mm <sup>2</sup>
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
Height with/without lid	5.3/3.7 mm
Growth area inner well	0.125 cm <sup>2</sup>
Coating area using 10 μl	0.23 cm <sup>2</sup>
Bottom	ibidi Polymer Coverslip

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## 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

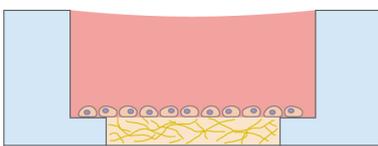
In tube formation assays the  $\mu$ -Slide 15 Well 3D is coated with a 0.8 mm thick layer of gel matrix.

1. Prepare your gel matrix according to the manufacturer's protocol or reference.
2. Fill the inner well with 10  $\mu$ l 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  $\mu$ l coating solution and calculate with an area to be coated of 0.23 cm<sup>2</sup> per well. Further information about coatings is provided in [Application Note 08 "Cell culture coating"](#).

## 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 50  $\mu$ l of the cell suspension into the upper well. Do not touch the gel matrix with the pipet tip.
3. Cover the  $\mu$ -Slide 15 Well 3D with the supplied lid. Incubate at 37°C and 5% CO<sub>2</sub> 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 50  $\mu$ l fresh medium.

For a detailed protocol please refer to [Application Note 19 "Tube Formation"](#) and [Application Note 5 "Tube Formation in  \$\mu\$ -Plate 96 Well 3D"](#).

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  $\mu$ -Slide 15 Well 3D before usage inside the incubator overnight.

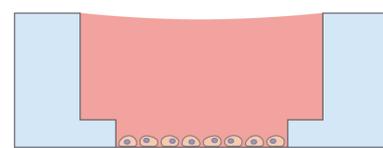
In case bent gel surfaces are created, increase or decrease the amount of gel used, until you get flat and even gels.

### Tip:

For less evaporation the space in-between the wells 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  $\sim 50^\circ\text{C}$ .

## Seeding Cells in 2D

You can also use the  $\mu$ -Slide 15 Well 3D for a standard 2D cell culture without gel matrix.

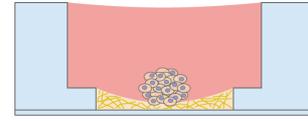


1. Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 1.8–

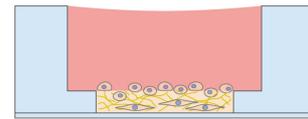
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- $4.3 \times 10^5$  cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 10 μl cell suspension into each well of the μ-Slide 15 Well 3D. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover the slide with the supplied lid. Incubate at 37°C and 5% CO<sub>2</sub> as usual.
- After cell attachment, add 50 μl cell-free medium to fill the upper 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.



### Attention!

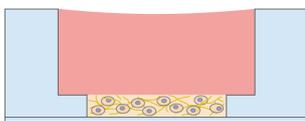
Avoid evaporation during seeding and cell culture in the incubator! We recommend placing the μ-Slide 15 Well 3D in an extra humidity chamber (e.g. a Petri Dish with wetted paper).

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 60 μl fresh medium per well.

## Experimental Setups

Alternatively, the μ-Slide 15 Well 3D can be used for the following assays:

- Fill the inner well with cells suspended inside a gel matrix. After gel polymerization, add 50 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 50 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.

## Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the μ-Slide 15 Well 3D. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on [ibidi.com](http://ibidi.com).

Chemical / Solvent	Compatibility
Methanol	yes
Ethanol	yes
Formaldehyde	yes
Acetone	yes, without lid
Mineral oil	no
Silicone oil	yes
Immersion oil	See <b>Immersion Oil</b> on page 4.

## 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 mounting media (50001 and 50011) optimized for μ-Dishes, μ-Slides, and μ-Plates.

### Note:

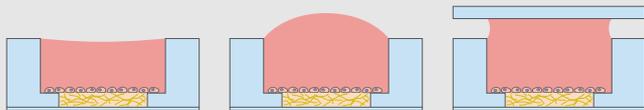
When gel matrices are used the optical quality and the use of high magnification objective lenses might be restricted.

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#### Tip:

For phase contrast imaging after the experiment, the upper well can be overfilled with additional 25  $\mu$ l. Closing the lid eliminates the meniscus of the upper well. This will create perfect phase contrast images. Please keep in mind that this overfilling technique might lead to well-to-well crosstalk. Therefore, we recommend this for final examination using phase contrast microscopy only.



## 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
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017
Leica	Immersion Liquid	11513859	n.a.	03/2011
Nikon	Immersion Oil F2 30cc	MXA22192	n.a.	01/2020
Nikon	Silicone Immersion Oil 30cc	MXA22179	20191101	01/2020
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Zeiss	Immersionol 518 F	444960	160706	01/2017
Zeiss	Immersionol W 2010	444969	101122	04/2012

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#### Ordering Information

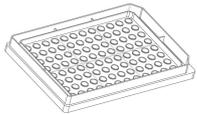
Our well-in-a-well family is available with different surfaces and in different formats. Please see the table below for choosing your μ-Slide or μ-Plate, respectively.

#### μ-Slide 15 Well 3D



Cat. No.	Description
81506	<b>μ-Slide 15 Well 3D ibiTreat:</b> #1.5 polymer coverslip, tissue culture treated, sterilized, individually packed
81501	<b>μ-Slide 15 Well 3D Uncoated:</b> #1.5 polymer coverslip, hydrophobic, sterilized, individually packed
81507	<b>μ-Slide 15 Well 3D Glass Bottom:</b> #1.5H (170 μm ±5 μm) D 263 M Schott glass, sterilized, individually packed

#### μ-Plate 96 Well 3D



Cat. No.	Description
89646	<b>μ-Plate 96 Well 3D ibiTreat:</b> #1.5 polymer coverslip, tissue culture treated, sterilized, individually packed

#### For research use only!

Further information can be found at [ibidi.com](http://ibidi.com). For questions and suggestions please contact us by e-mail [info@ibidi.de](mailto:info@ibidi.de) or by telephone +49 (0)89/520 4617 0.

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