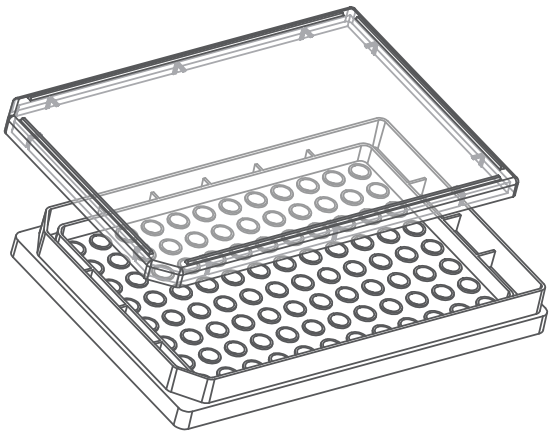
µ-Plate 96 Well 3D

Instruction Manual



This document applies to the following product:

89646	**µ-Plate 96 Well 3D ibiTreat**

The ibidi labware 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 ibidi Polymer Coverslip is similar to that of glass, enabling a variety of microscopy techniques with uncompromised resolution and choice of wavelength.
With its "well-in-a-well" technology, the µ-Plate 96 Well 3D has a specialized geometry for the easy, convenient, and reproducible conduction of tube formation assays. It is also ideal for sprouting assays, immunofluorescence staining, and 3D cell culture.

Material

The µ-Plate 96 Well 3D is made of a polymer that has the highest optical quality. The ibidi Polymer Coverslip bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. It is not possible to detach the bottom from the upper part. The plate is intended for one-time use and is not autoclavable, since it is 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 of Polymer Coverslip	
Refractive index (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	Polymer

WARNING – The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found in the Section "Immersion Oil".

Shipping and Storage

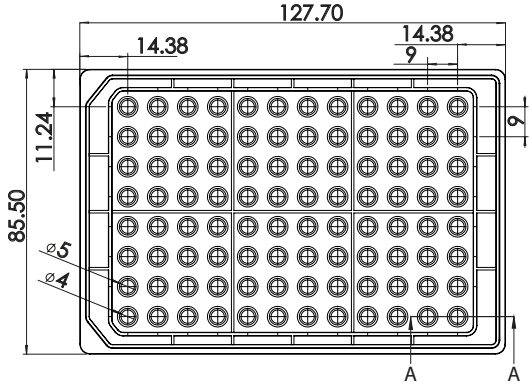
This product is sterilized and sealed in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

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

Shelf Life	
ibiTreat	36 months

Geometry

The µ-Plate 96 Well 3D provides standard geometry and numbering (A–H, 1–12).

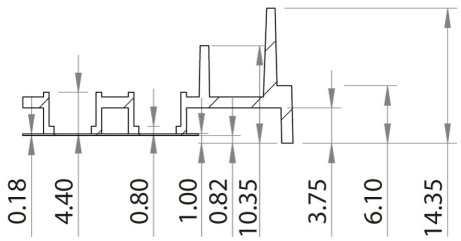


The µ-Plate 96 Well 3D meets all important values of the ANSI/SLAS (SBS) Standards (1-2004, 2-2004, 3-2004 and 4-2004).

Every well of the µ-Plate 96 Well 3D consists of an inner and an upper well. This "well-in-a-well" technology reduces gel volumes to 10 µl per well, and no gel meniscus is formed.

Specifications (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 Dimensions	
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
Focal offset	1 mm



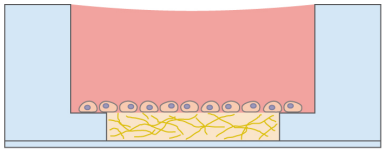
Section A - A

Surface

The µ-Plate 96 Well 3D is available with an ibiTreat surface. The tissue culture-treated, hydrophilic ibiTreat surface of the ibidi Polymer Coverslip is ideal for culturing adherent cells. It ensures excellent cell adhesion without the necessity for any additional coatings. Nonetheless, extracellular matrix (ECM) protein coatings can be applied to the ibiTreat surface without any restrictions, if required.

Tube Formation Assay

In tube formation assays, the inner wells of the µ-Plate 96 Well 3D are filled with a thick layer of gel matrix. Cells are seeded on top of the gel matrix:



For a more detailed protocol, please refer to Application Note 05: Tube Formation Assay in the µ-Plate 96 Well 3D.

An example experiment for a tube formation assay using Laminin-Collagen I gel matrix in the µ-Slide 15 Well 3D can be found in the Application Note 66: Tube Formation Assay With Laminin-Collagen I Gel in the µ-Slide 15 Well 3D.

Further information about assay optimization and data analysis is provided in Application Note 27: Optimizing Tube Formation Assays and Application Note 70: Data Analysis of Tube Formation Assays.

1. Prepare your gel matrix according to the manufacturer's specifications.
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. If storage is needed, fill the area around the wells with sterile water to create a humidified environment and prevent evaporation.
5. 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.
6. Apply 70 µl of the cell suspension into the upper well. Do not touch the gel matrix with the pipet tip.

7. Cover the µ-Plate 96 Well 3D with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2).

8. 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 per well.

TIP – Air bubbles in the gel can be reduced by equilibrating the µ-Plate 96 Well 3D in the incubator overnight before use. If bent gel surfaces are formed, adjust the amount of gel used until you achieve flat and even gels.

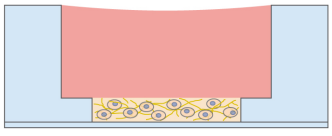
TIP – To reduce evaporation, fill the reservoirs at the edges with sterile water or agarose. To prepare the agarose solution, add agarose to water or buffer solution (e.g., 0.1 g to 10 ml water). Melt the agarose solution using a microwave or boiling water bath, then allow it to cool to approximately 50 °C before use.

TIP – You can stack the µ-Plates to save space in your incubator. This will not affect cell growth. Due to stability reasons, we recommend making batches with not more than 6 plates.

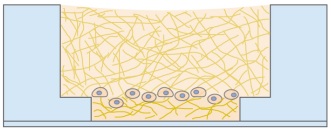
3D Cell Culture Applications

Alternatively, the µ-Plate 96 Well 3D can be used for the following 3D cell culture assays:

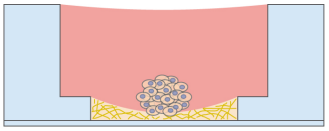
- 3D cell culture in a gel matrix: Fill the inner well with cells suspended inside a gel matrix. After gelation, add 70 µl cell-free medium to fill the upper well.



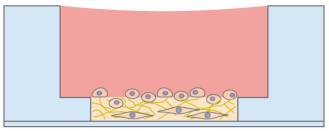
- Sandwich cell culture: Fill the inner well with a gel matrix. Seed cells on top of the gel matrix and embed the cells with 70 µl gel in the upper well.



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



- Co-culture assay: Fill the inner well with fibroblasts suspended inside a gel matrix. Seed cells on top of the gel. Overlay the cell layer with medium and incubate to analyze cell invasion into the gel matrix.



Coating

Non-gel-based coatings are possible when using the µ-Plate 96 Well 3D.

Detailed information about coatings is provided in Application Note 08: Coating Protocols for ibidi Labware.

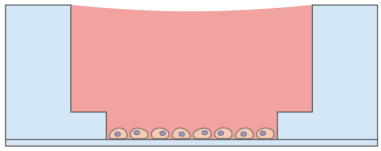
In short, specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 0.23 cm^2 and a volume of 10 µl per well.

2. Apply 10 µl per well and leave it at room temperature for at least 30 minutes.
3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. The coated plate is ready to be used. Be aware that allowing the coated surface to dry out is not recommended, as some coating proteins may degrade upon drying.

Seeding Cells in 2D

You can also use the µ-Plate 96 Well 3D for a low-volume 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 96 Well 3D. Avoid shaking as this will result in inhomogeneous distribution of the cells.
3. Cover the slide with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2).
4. After cell attachment, add 70 µl cell-free medium to fill the upper well.

CAUTION – To avoid evaporation during seeding and cell culture in the incubator, we recommend placing the µ-Plate 96 Well 3D in an additional humidity chamber, such as a Petri dish with wetted paper.

Insensitive cells can be left in their seeding medium for several days and grow to confluence there. However, optimal results might be achieved when the medium is changed every 1–2 days. For this, carefully aspirate the old medium and replace it by up to 80 µl fresh medium.

Microscopy

To image your cells, no special preparations are necessary. Living or fixed cells can be directly observed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and for storage of fixed and stained samples, ibidi provides mounting media that are optimized for ibidi labware:
Cat. No. 50001: ibidi Mounting Medium
Cat. No. 50011: ibidi Mounting Medium with DAPI

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

Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the µ-Plate 96 Well 3D. For a full list of compatible solvents and more information on chemical compatibility, visit ibidi.com/chemicals.

Chemical / Solvent	Compatibility
Methanol	Yes
Ethanol	Yes
Formaldehyde	Yes
Acetone	Yes, without lid
Mineral oil	No
Silicone oil	Yes
Immersion oil	See Section “Immersion Oil”

Immersion Oil

WARNING – 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 2	50102	24-07-04	07/2024
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.	03/2023
Leica	Immersion Liquid Type G	11513910	n.a.	04/2024
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	Immersol 518 F	444960-0000	220211	03/2023
Zeiss	Immersol 518 F (30 ℃)	444970-9010	220816	03/2023
Zeiss	Immersol 518 F (37 ℃)	444970-9000	220302	03/2023
Zeiss	Immersol W 2010	444969-0000	101122	04/2012
Zeiss	Immersol Sil 406	444971-9000	80730	03/2023
Zeiss	Immersol G	462959-9901	211117	03/2023

For research use only!

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