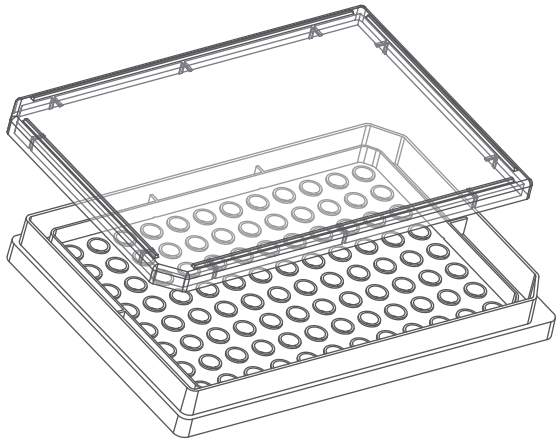
µ-Plate 96 Well 3D Glass Bottom

Instruction Manual



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 glass bottom versions are especially designed for TIRF, super-resolution, and single molecule applications.
With its “well-in-a-well” technology, the µ-Plate 96 Well 3D Glass Bottom 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.

This document applies to the following product:

89647	**µ-Plate 96 Well 3D Glass Bottom**

Material

The µ-Plate 96 Well 3D Glass Bottom is made with a glass coverslip bottom. 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.

Optical Properties Glass Coverslip	
Refractive index	1.523
Abbe number	55
Thickness	No. 1.5H (170 µm ± 5 µm)
Material	Schott borosilicate glass, D 263 M

CAUTION – Be cautious when handling ibidi labware products with a glass bottom! The glass coverslip or slide is fragile and can break easily. Handle these items carefully to prevent physical injury and damage to devices due to medium leakage.

Shipping and Storage

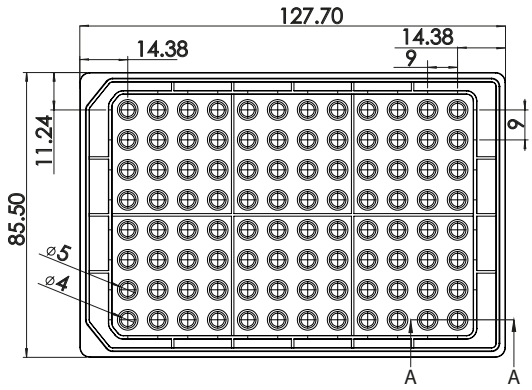
This product is sterilized and sealed in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

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

Shelf Life	
Glass Bottom	36 months

Geometry

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



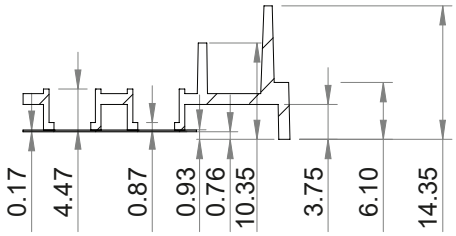
The µ-Plate 96 Well 3D Glass Bottom meets all important values of the ANSI/SLAS (SBS) Standards (1-2004, 2-2004, 3-2004 and 4-2004).

Every well of the µ-Plate 96 Well 3D Glass Bottom 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	0.87 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	0.76 mm
Focal offset	0.93 mm
Bottom	Glass



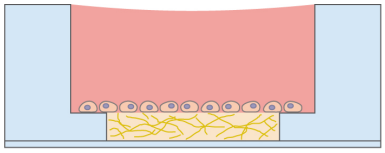
Section A - A

Surface

The µ-Plate 96 Well 3D Glass Bottom is manufactured with an uncoated glass coverslip. Washing it (e.g., with PBS) before cell seeding helps removing glass dusts, which enhances direct cell growth on the surface.

Tube Formation Assay

In tube formation assays, the inner wells of the µ-Plate 96 Well 3D Glass Bottom 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 Glass Bottom 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 Glass Bottom 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.

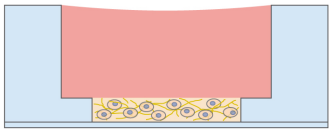
TIP – To reduce evaporation, fill the reservoirs at the edges with sterile water or agarose. To prepare the agarose solution, add agarose to water or buffer solution (e.g., 0.1 g to 10 ml water). Melt the agarose solution using a microwave or boiling water bath, then allow it to cool to approximately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

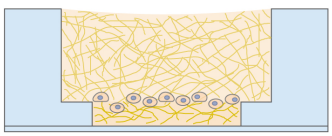
3D Cell Culture Applications

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

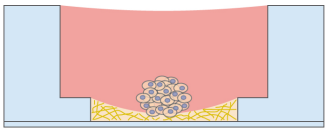
- 3D cell culture in a gel matrix: Fill the inner well with cells suspended inside a gel matrix. After gelation, add 70 µl cell-free medium to fill the upper well.



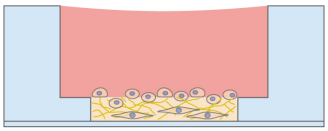
- Sandwich cell culture: Fill the inner well with a gel matrix. Seed cells on top of the gel matrix and embed the cells with 70 µl gel in the upper well.



- 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 Glass Bottom.

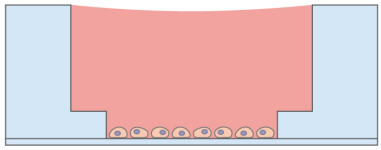
Detailed information about coatings is provided in Application Note 08: Coating Protocols for ibidi Labware.

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 Glass Bottom 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 Glass Bottom. 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 Glass Bottom 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

Immersion Oil

When using ibidi Glass Bottom products with oil immersion objectives, there is no known incompatibility with any immersion oil on the market. All types of immersion oils can be used.

Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the µ-Plate 96 Well 3D Glass Bottom. 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	No
Mineral oil	Yes
Silicone oil	Yes
Immersion oil	See Section “Immersion Oil”

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.