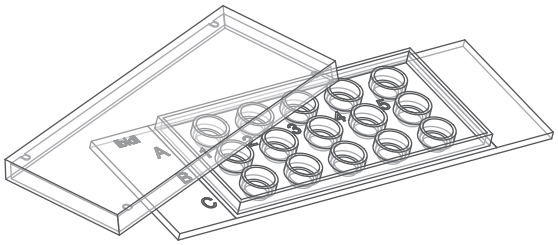
µ-Slide 15 Well 3D Glass Bottom

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-Slide 15 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 general 3D cell culture.

This document applies to the following product:

81507	**µ-Slide 15 Well 3D Glass Bottom**

Material

The µ-Slide 15 Well 3D Glass Bottom is made with a glass coverslip bottom. It is not possible to detach the bottom from the upper part. The slide is intended for one-time use and is not autoclavable, since it is only temperature-stable up to 80 °C/175 °F.

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

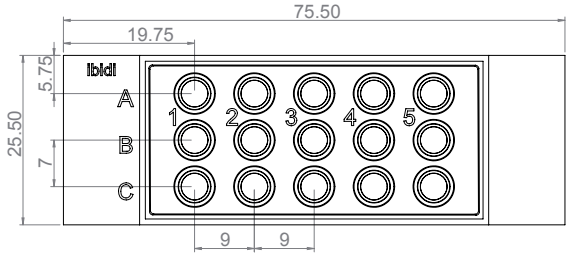
CAUTION – Be cautious when handling ibidi labware products with a glass bottom! The glass coverslip or slide is fragile and can break easily. Handle these items carefully to prevent physical injury and damage to devices due to medium leakage.

Surface

The µ-Slide 15 Well 3D Glass Bottom is manufactured with a glass coverslip. Washing it (e.g., with PBS) before cell seeding helps removing glass dusts, which enhances direct cell growth on the surface.

Geometry

The µ-Slide 15 Well 3D Glass Bottom provides standard slide format according to ISO 8037/1.



Every well of the µ-Slide 15 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	
Outer dimensions (w × l)	25.5 × 75.5 mm^2
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^2
Coating area using 10 µl	0.23 cm^2
Bottom	Glass Bottom

Shipping and Storage

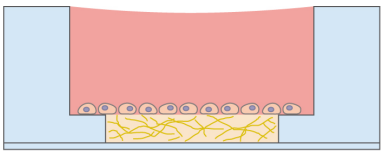
This product is sterilized and sealed in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

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

Shelf Life	
Glass Bottom	36 months

Tube Formation Assay

In tube formation assays, the inner wells of the µ-Slide 15 Well 3D Glass Bottom are filled with a 0.8 mm thick layer of gel matrix. Cells are seeded on top of the polymerized gel matrix:



For a detailed protocol please refer to Application Note 19: Tube Formation Assay in the µ-Slide 15 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 50 µl of the cell suspension into the upper well. Do not touch the gel matrix with the pipet tip.
7. Cover the µ-Slide 15 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 50 µl fresh medium per well.

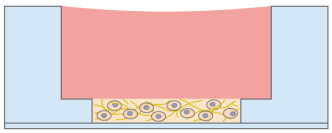
TIP – To minimize air bubbles in the gel, equilibrate the µ-Slide 15 Well 3D Glass Bottom in the incubator overnight before use. If curved gel surfaces form, adjust the gel volume to achieve flat and uniform layers.

CAUTION – To avoid evaporation during seeding and cell culture in the incubator, we recommend placing the µ-Slide 15 Well 3D Glass Bottom in an additional humidity chamber, such as a Petri dish with wetted paper.

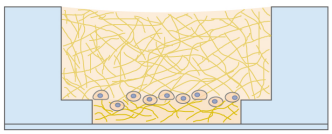
3D Cell Culture Applications

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

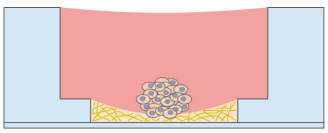
- 3D cell culture in a gel matrix: Fill the inner well with cells suspended inside a gel matrix. After gelation, add 50 µl cell-free medium to fill the upper well.



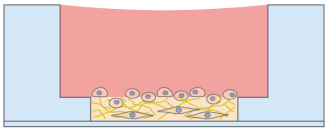
- Sandwich cell culture: Fill the inner well with a gel matrix. Seed cells on top of the gel matrix and embed the cells with 50 µl gel in the upper well.



- 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-Slide 15 Well 3D Glass Bottom.

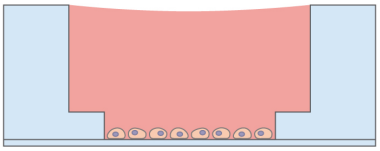
Detailed information about coatings is provided in Application Note 08: Coating Protocols for ibidi Labware.

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 slide 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-Slide 15 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-Slide 15 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 50 µl cell-free medium to fill the upper well.

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 60 µl fresh medium.

Chemical Compatibility

The following table provides basic information on the chemical and solvent compatibility of the µ-Slide 15 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”

Immersion Oil

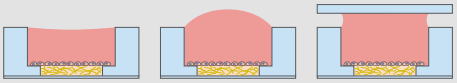
When using ibidi Glass Bottom products with oil immersion objectives, there is no known incompatibility with any immersion oil on the market. All types of immersion oils can be used.

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.

TIP – For phase contrast imaging after the experiment, the upper well can be overfilled with additional 25 µl. 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.



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.