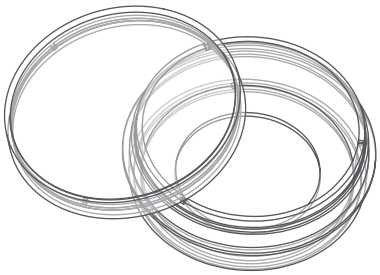
µ-Dish 35 mm, high Bioinert

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 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.

The surface of the µ-Dish 35 mm, high Bioinert is completely non-adherent and allows no binding of any biomolecule, even in long-term experiments. This makes Bioinert ideal for the culture and high-resolution imaging of suspension cells and cell aggregates, such as spheroids, organoids, and embryoid bodies.

This document applies to the following product:

81150	**µ-Dish 35 mm, high Bioinert**

Material

The µ-Dish 35 mm, high Bioinert 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 dish 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), dry place (relative humidity <50%)

Shelf Life	
Bioinert	36 months

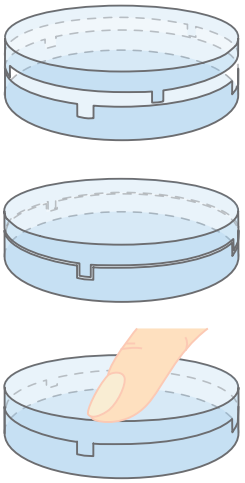
Geometry

Specifications	
⌀ dish	35 mm
Volume	2 ml
Growth area	3.5 cm^2
⌀ observation area	21 mm
Height with / without lid	14 mm / 12 mm
Bottom	ibidi Polymer Coverslip
Bioinert surface thickness	200 nm
Bioinert surface material	Polyol-based hydrogel
Protein coatings	Not possible

Surface

The Bioinert surface is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip. It allows no adsorption, coating, or binding of proteins, antibodies, enzymes, and other biomolecules. Therefore, the Bioinert technology provides a stable passivation in cell-based assays for several days or even weeks. The hydrophilic Bioinert surface hinders any protein attachment, thus inhibiting subsequent cell attachment. The Bioinert surface is not biodegradable by cells allowing long-term assays with suspension cells and cell aggregates, such as spheroids, organoids, and embryoid bodies.

Lid with Locking Feature for Minimized Evaporation



Open position for easy opening

Closed position for cell cultivation with minimal evaporation

Lock position for long-term studies with almost no evaporation

Seeding Cells

Without a surface modification, Bioinert does not support direct cell adherence. Depending on your application the number of cells or cell aggregates might differ. Follow these steps for a general cell application protocol:

1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, we recommend a 4–9 × 10^4 cells/ml suspension.
2. Apply 400 µl cell suspension into the growth area of the dish. Avoid shaking, as this will result in inhomogeneous cell distribution.
3. After the cells have settled down, add 1.6 ml of medium to ensure optimal grow conditions. Add the medium carefully, touching the side walls with the pipet tip.
4. Cover the dish with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2).

CAUTION – Due to the large and flat imaging area, spheroid formation in the µ-Dish 35 mm, high Bioinert is different compared to standard techniques. Depending on the cell type, one single or multiple spheroids will be formed over time.

CAUTION – Make sure to avoid uneven incubator shelves and microscope stages. Single cells or cell clusters might roll on one side over time. Please also avoid evaporation and temperature changes. Both will lead to convectional flow.

TIP – You can stack the µ-Dishes 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 µ-Dishes. Placing the µ-Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination each time the incubator is opened.

Transfer of Spheroids or Cell Aggregates

1. Harvest spheroids and transfer them into the inner well of the µ-Dish using a volume of 400 µl.

2. After the spheroids have settled down, fill the entire µ-Dish with 1.6 ml of culture medium. Add the medium carefully touching the side walls with the pipet tip.
3. Cover the dish with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO_2).

Medium Exchange

Since the Bioinert surface does not allow any cell adhesion, washing out the cells or cell clusters has to be avoided. Please follow these steps for an easy medium exchange:

1. Gently swirl the µ-Dish 35 mm, high Bioinert. This will focus the cells in the center.
2. Wait for 10 seconds to let the cells settle down.
3. Slowly and carefully aspirate 1.6 ml of the old medium touching the side wall with the pipet tip. Do not aspirate the central observation area.
4. Gently refill the dish with 1.6 ml of culture medium. Add the medium carefully from the side wall.

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. Due to the thin bottom, high-resolution microscopy is possible.

Without disturbing effects such as convection, evaporation, and fast stage accelerations, there is no need to stabilize your cell samples on the Bioinert surface. Minimize convectional flow caused by evaporation or temperature gradients. Even without cell attachment, single cells, spheroids, or other clusters remain stable on the Bioinert surface.

Optional: Agarose Fixation for Live Cell Imaging

For a maximum of stability, 1% agarose (low melt) can be used to increase the culture medium's viscosity. This will lead to less Brownian motion and improved imaging conditions. Please follow this protocol:

1. Prepare approximately 5 ml of a 3% agarose (low melt) solution in culture medium or buffer using a 50 ml Falcon tube. Heat up according to the manufacturer's protocol to dissolve the agarose completely.
2. Adjust the agarose solution to 37 °C and spin down to remove air bubbles.
3. Carefully aspirate 1.6 ml of the culture medium from the µ-Dish 35 mm, high Bioinert as described in the Section "Medium Exchange".
4. Transfer the remaining 400 µl containing the cells/spheroids from the inner observation area to a reaction tube. Use an appropriate sized pipet tip to avoid damaging larger spheroids by the small opening of the pipet tip.
5. Gently mix 200 µl of the agarose solution with the 400 µl cell suspension. Make sure to avoid air bubbles.
6. Pipet the mixture into the central observation area of the µ-Dish 35 mm, high Bioinert.
7. Refill the dish with cell culture medium to a maximal volume of 2 ml.

Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the µ-Dish 35 mm, high Bioinert. 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.