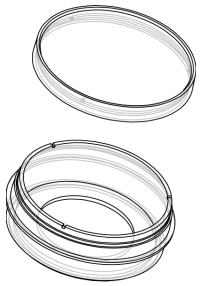


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

The Bioinert surface is a thin hydrogel layer that is covalently attached to the ibidi Polymer Coverslip No. 1.5. In contrast to standard ultra-low attachment (ULA) coatings, 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, like spheroids, organoids and embryoid bodies.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates are made of a plastic 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 not autoclavable, since they are only temperature–stable up to 80°C/175°F. Please note that gas exchange between the medium and 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 3.

The Bioinert Surface

The Bioinert surface 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. Unlike with the ibiTreat and Uncoated surfaces, a coating is not possible.

Geometry

Geometry of the µ–Dish 35mm, high Bioinert	
Diameter dish	35 mm
Volume	2000 µl
Growth area	3.5 cm^2
Diameter observation area	21 mm
Height with / without lid	14 mm / 12 mm
Bottom matches coverslip	No. 1.5
Bioinert surface thickness	200 nm
Bioinert surface material	Polyol-based hydrogel
Protein coatings	Not possible

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.

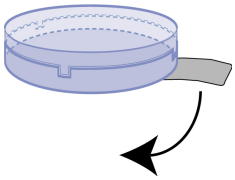
Conditions	
Shipping conditions	Ambient
Storage conditions	RT (15-25°C)

Shelf Life	
Bioinert surface	6 months

Store the Bioinert products in a dry place (relative humidity <50%) for maintaining the maximum shelf life. High humidity (relative humidity >50%) might reduce the shelf life down to 4 weeks.

Protection Film

Remove the protection film before usage!



The bottom of the µ–Dish is covered with a film to protect the optical quality of the surface. Please pull off the protection film before usage!

Cell Seeding - Single Cells

- Detach and count cells as usual. Dilute the cell suspension to a concentration of 4–9 × 10^4 cells/ml.
- Apply 400 µl cell suspension into the inner well of the µ–Dish. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- After the cells 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.
- Cover the µ–Dish with the supplied lid. Incubate at 37°C and 5 % CO_2 as usual.

Note:

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.

Transfer of Spheroids or Cell Aggregates

- Harvest spheroids and transfer them into the inner well of the µ–Dish using a volume of 400 µl.
- 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.
- Cover the µ–Dish with the supplied lid. Incubate at 37°C and 5 % CO_2 as usual.

Medium Exchange

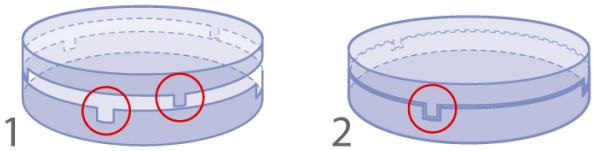
Since the Bioinert surface does not allow any cell adhesion, washing out the cells or cell clusters has to be avoided. Please follow these steps for an easy medium exchange:

- Gently swirl the µ–Dish 35 mm, high Bioinert. This will focus the cells in the center.
- Wait for 10 seconds to let the cells settle down.
- Carefully aspirate the old medium from all four corners. Remove 400 µl from each corner near the side wall. Do not aspirate the central observation area.
- Gently refill the entire µ–Dish with 1.6 ml of culture medium. Add the medium carefully, touching the side walls with the pipet tip.

Tip:

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.

Using The Lid



1. Open position, easy opening
2. Close position, for long term studies, minimal evaporation

Preparation for Cell Microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Dish 35 mm, high Bioinert preferably on an inverted microscope. You can use any fixative of your choice. The µ–Dish 35 mm, high Bioinert material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom high resolution microscopy is possible.

Without disturbing effects like convection, evaporation and fast stage accelerations, there is no need to stabilize your cell samples on Bioinert. Minimize convectional flow

caused by evaporation or temperature gradients. Even without cell attachment, single cells, spheroids or other clusters will sit on the Bioinert surface in a stable way.

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 better imaging conditions. Please follow this protocol:

- 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.
- Adjust the agarose solution to 37°C and spin down to remove air bubbles.
- Carefully remove 1.6 ml of the culture medium from the µ–Dish 35 mm, high Bioinert similar to the medium exchange protocol.
- Remove the remaining 400 µl with the cells/spheroids from the inner observation area. Make sure to use an appropriate sized pipet tip, not to destroy larger spheroids by the small opening of the pipet tip.
- Gently mix 200 µl of the agarose solution with the 400 µl cell suspension. Make sure to avoid air bubbles.
- Refill the mixture into the central observation area of the µ–Dish 35 mm, high Bioinert.

Tip:

You can stack the µ–Dishes to save space in your incubator. This will not affect cell growth. We recommend making batches with up to 6 µ–Dishes, due to stability reasons. Placing the µ–Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination when the incubator is opened.

Minimizing Evaporation

Using the µ–Dish with a closed lid, the evaporation in an incubator system with 37°C and 95 % humidity is around 1 % per day. Using the µ–Dish with a closed lid in a 37°C heating system with low humidity (between 20 % and 40 %), the evaporation is around 10 % per day. For reducing the evaporation down to 1 % per day in all systems, we recommend sealing the lid with ibidi Anti–Evaporation Oil (50051).

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

For research use only!

Further technical specifications can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0. All products are developed and produced in Germany.