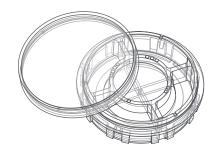


μ-Dish ^{35 mm} Quad Instruction Manual



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 μ -Dish $^{35~mm}$ Quad allows you to perform high-resolution microscopy in a 35 mm Petri dish with a 4 well subdivision. The Ph⁺ structure in the center enables excellent phase contrast microscopy without any distracting meniscus. The lid can be closed to hinder evaporation during long-term experiments.

This document applies to the following products:

| 80416 | μ-Dish 35 mm | Quad | ibiTreat |
|-------|-------------------------|------|----------|
| 80411 | μ-Dish ^{35 mm} | Quad | Uncoated |

Material

The μ -Dish $^{35\,\text{mm}}$ Quad 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 $80\,^{\circ}\text{C}/175\,^{\circ}\text{F}$. 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 CoverslipRefractive index (589 nm)1.52Abbe number56ThicknessNo. 1.5 (180 μm)MaterialPolymer



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

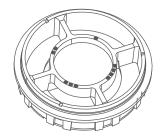
This product is sterilized and sealed in a gaspermeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

| Conditions | | | | | | |
|---------------------|--------------|--|--|--|--|--|
| Shipping conditions | Ambient | | | | | |
| Storage conditions | RT (15–25°C) | | | | | |
| | | | | | | |
| Shelf Life | | | | | | |
| ibiTreat, Uncoated | 36 months | | | | | |
| | | | | | | |

Geometry

| Specifications | | | | | |
|--------------------------|----------------------|--|--|--|--|
| ∅ dish | 35 mm | | | | |
| Volume per well | 300 μl | | | | |
| Liquid height | 4.0 mm | | | | |
| Growth area per well | 0.85 cm ² | | | | |
| Coating area per well | 2.46 cm ² | | | | |
| Partition wall | 0.6 mm | | | | |
| ø growth area | 21 mm | | | | |
| Height with/without lid | 12 mm/10 mm | | | | |
| Bottom matches coverslip | No. 1.5 | | | | |

The wells are labelled by small points on the edge of the small plate to guarantee a definite identification:



Surface

The μ -Dish $^{35 \text{ mm}}$ Quad is available with either an ibiTreat or an Uncoated 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.

The hydrophobic Uncoated surface of the ibidi Polymer Coverslip offers weak cell adhesion unless pre-coated with an ECM protein. You can apply coatings to the Uncoated surface without any restrictions. This surface is suitable for culturing adherent cells that require a specific coating.

For establishing a particular coating, we advise testing your procedure on both ibiTreat and Uncoated surfaces, as proteins and biomolecules may adhere differently to hydrophilic or hydrophobic surfaces.



TIP – The day before seeding the cells we recommend placing the cell medium and the μ -Dish ^{35 mm} Quad into the incubator for equilibration. This will prevent the liquid inside wells from emerging air bubbles over the incubation time.

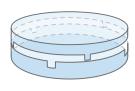
Coating

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

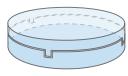
In short, specific coatings are possible following this protocol:

- Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 2.46 cm² and a volume of 300 µl per well.
- 2. Apply 300 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 dish 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.

Lid with Locking Feature for Minimized Evaporation



Open position for easy opening



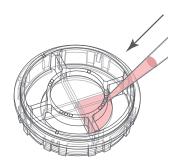
Closed position for cell cultivation with minimal evaporation



Lock position for long-term studies with almost no evaporation

Seeding cells

- Trypsinize and count the cells as usual and dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 1.3–2.7 x 10⁴ cells/ml suspension should result in a confluent layer within 2–3 days.
- 2. Apply 300 μl cell suspension into each well of the μ-Dish^{35 mm} Quad. If the pipet tip does not reach the bottom of each well as depicted in the image below, air bubbles may form and remain beneath the center plate of the wells. To prevent the formation of air bubbles, use a gel loader pipet tip, which has a thin and flexible end that can reach the bottom of the well.



- 3. Avoid shaking, as this will result in inhomogeneous cell distribution.
- 4. After cell attachment, add $300\,\mu l$ of medium to ensure optimal growing conditions.
- 5. Cover the μ -Dish with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% CO₂).

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 2–3 days. For this, carefully aspirate the old medium and replace it by up to $600\,\mu l$ fresh medium.



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.

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



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 °C) | 444970-9010 | 220816 | 03/2023 |
| Zeiss | Immersol 518 F (37°C) | 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 |

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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.

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