

Instructions

u-Dish 35mm, high ESS



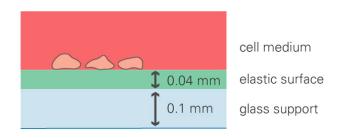
The ibidi product family is comprised of a variety of μ –Slides and μ –Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised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

The μ –Dish $^{35mm, \, high}$ allows you to perform high resolution microscopy in a 35 mm Petri–dish with 12 mm walls. The standard height allows convenient liquid handling. The lid can be closed to hinder evaporation during long term experiments.

The ibidi μ –Dish ^{35mm , high ESS is a product for growing cells on a *in vivo*–near elastic surface. ESS stands for elastically supported surface. The surface elasticity (Young Module) is 1.5 kPa, 15 kPa, or 28 kPa.

Material

The μ –Dish ESS provides a special elastic surface for *in vivo* like cell cultivation. On a 100 μ m thin glass cover slip, a 40 μ m highly-elastic material (polydimethylsiloxane) is coated. The whole bottom provides a thickness of 140 μ m and a very high optical quality.



Geometry

| Geometry of the μ–Dish ^{35 mm, high} ESS | | |
|---|--------------------|--|
| Diameter dish | 35 mm | |
| Volume | 2000 µl | |
| Growth area | 3.5 cm^2 | |
| Diameter growth area | 21 mm | |
| Coating area using 800 µl | 4.2 cm^2 | |
| Height with / without lid | 14 mm / 12 mm | |
| Bottom matches coverslip | No. 1 (140 μm) | |

Attention!

Be cautious when handling μ –Slides and μ –Dishes with glass bottom! The glass coverslip is very fragile and might break easily. Handle with care to avoid physical injury and damage to devices through leakage of the medium.

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 of Different Surfaces | | |
| ibiTreat, Glass Bottom, ESS | 36 months | |
| Collagen, Poly-Lysine | 18 months | |
| Fibronectin | 4 months | |

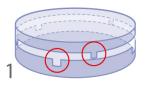
Surface and coating

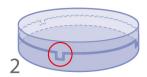
The uncoated μ –Dish $^{35mm,\ high}$ ESS must be coated to promote cell adhesion. Please follow these steps or see Application Note 08 "Cell culture coating" on www.ibidi.com.

- Prepare your coating solution according to the manufacturer's specifications or reference. Adjust the concentration to a coating area of 4.2 cm² and 800 µl.
- Apply 800 μ l into the growth area. Make sure that the entire bottom is covered with liquid easily tilting or shaking the μ -Dish. Put on the lid and leave at room temperature for at least 60 minutes.
- Aspirate the solution and wash. Use as soon as possible. The ESS surface must not be dried after coating.



Using The Lid





- 1. open position, easy opening
- 2. close position, for long term studies, minimal evaporation

Seeding Cells

Depending on your cell type, application of a 4–9 \times 10^4 cells/ml suspension should result in a confluent layer within 2–3 days.

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration.
- 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 cell attachment add additionally 1.6 ml of pure medium to ensure optimal grow conditions.
- Cover the μ –Dish with the supplied lid. Incubate at 37°C and 5 % CO₂ as usual.

We recommend not to fill more than the indicated total volume into the μ -Dish $^{35\text{mm}, \text{ high}}$ ESS in order to avoid the liquid contacting the lid.

Undemanding cells can be left in their seeding medium for several days and grow to confluence there. However, best results are achieved when the medium is changed every 2–3 days. Carefully aspirate the old medium and replace it by up to 2 ml fresh medium.

Preparation for cell microscopy

Using ESS it is possible to combine microscopy with a close to nature environment. To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the μ –Dish on an inverted microscope. For ESS μ –Dishes we recommend paraformaldehyde fixation. Due to the thin bottom of 140 μ m, high-end microscopy (confocal techniques, high resolution fluorescence, etc.) is possible.

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, use only the immersion oils specified in the table. The use of a non-recommended oil could lead to the damage of the plastic material and the objective.

| Company | Product | Ordering Number |
|---------|------------------|------------------|
| Zeiss | Immersol 518 F | (Zeiss) 444960 |
| Zeiss | Immersol W 2010 | (Zeiss) 444969 |
| Leica | Immersion liquid | (Leica) 11513859 |



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ESS family

The ESS surface is available with different surface elasticities in the $\mu\text{-Dish}$ $^{35mm,\,high}.$

μ-Dish ^{35mm, high} ESS



| Cat. No. | Description | Characteristics |
|----------|--|-------------------------|
| 81291 | μ –Dish ^{35mm, high} ESS 1.5 kPa Uncoated: \emptyset 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 1.5 kPa | hydrophobic, sterilized |
| 81391 | μ –Dish ^{35mm, high} ESS 15 kPa Uncoated: \emptyset 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 15 kPa | hydrophobic, sterilized |
| 81191 | μ –Dish ^{35mm, high} ESS 28 kPa Uncoated: \emptyset 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 28 kPa | hydrophobic, sterilized |
| 81199 | μ –Dish ^{35mm, high} ESS Variety Pack Uncoated: \emptyset 35 mm, high wall (2 ml volume), elastic surface with a stiffness of 1.5, 15, and 28 kPa | hydrophobic, sterilized |



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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. © ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.