Instructions





The ibidi product family comprises a variety of different shapes of µ-Slides and µ-Dishes which all have 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 ^{35 mm, 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 µm thin glass cover slip, a 40 µm highly-elastic material (polydimethylsiloxane) is coated. The whole bottom provides a thickness of 140 µm and a very high optical quality.



elastic surface

glass support

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)		

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 cm^2 and $800 \text{ }\mu\text{l}$.

- Apply 800 µl 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 30 minutes.
- Aspirate the solution and wash. Optionally, let dry at room temperature.

Using the lid



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

Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. 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.
- 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.

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.

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.

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 paraformalde-hyde 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, only the immersion oils specified in the table may be used. The use of different oil can lead to damages 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

ESS family

The ESS surface is available with different surface elasticities in μ -Dish ^{35mm, high}.

 μ -Dish ^{35mm, high} with ESS

Ordering number	Treatment or Coating	Characteristics
81291	elastic surface ESS, 1.5 kPa, uncoated, sterile	hydrophobic
81391	elastic surface ESS, 15 kPa, uncoated, sterile	hydrophobic
81191	elastic surface ESS, 28 kPa, uncoated, sterile	hydrophobic

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 46 17 0. All products are developed and produced in Germany. © ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.