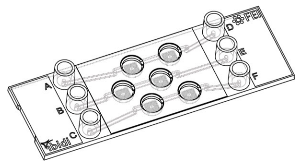
ibidi cells in focus



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–Slide CorrSight™ Live is an array of 6 wells where cells can be cultivated and, subsequently, investigated with microscopical methods. Two of the 6 wells respectively are connected by a channel. The channels can be connected to a pump in order to perfuse cells. Each of the wells contains a Grid–100 structure on its bottom. The Grid–100 is a grid structure for relocating events, based on the high quality ibidi Standard Bottom. It provides 100 distinguishable observation squares of 100 μm edge length. The grid is clearly visible by phase contrast microscopy and electron microscopy.

The μ–Slide CorrSight™ Live was designed for correlative light and electron microscopy (CLEM) with FEI's CorrSight™ microscope. It is also compatible with other CLEM applications or light microscopy applications such as perfusion of small samples, etc.

Material

ibidi μ–Slides, μ–Dishes, and μ–Plates are made of a plastic that has the highest optical quality. The bottom material 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 Standard Bottom	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 μm)
Material	microscopy plastic/ polymer coverslip

Please note! The ibidi Standard Bottom is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 4.

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

Geometry

The μ–Slide CorrSight™ Live provides a standard slide format according to ISO 8037/1.

Geometry of μ–Slide CorrSight™ Live	
Number of wells	6
Volume of wells	30 μl
Well diameter	5.5 μm
Growth area per well	25 mm^2
Number of channels	3
Total channel volume	130 μl
Channel width	1.0 mm
Adapters	Female Luer
Volume per reservoir	60 μl
Coating area using 130 μl	2.4 cm^2 per channel
Bottom matches coverslip	No. 1.5

Characteristics of the Grid

The Grid–100 is made of small dots inside the ibidi plastic surface of in the wells. The structure is imprinted on the side on which cells are growing and does not effect cell growth, coating protocols, or surface properties. Proliferation and cell behavior is comparable with standard non–gridded dishes. Cells and grid are in one focal plane.

Important!

This µ–Slide is hydrophobic, uncoated. For all adherent cells, a coating is necessary.

Geometry of the Grid–100

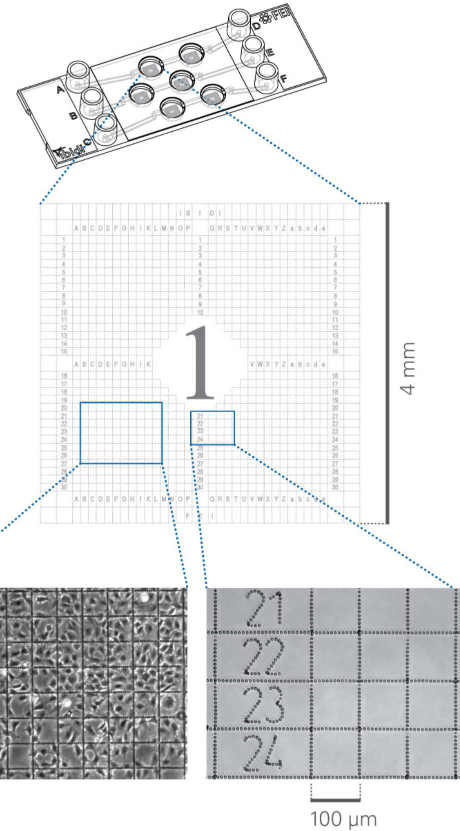
The µ–Slide CorrSight™ Live provides a standard slide format according to ISO 8037/1.

Geometry of the Grid–100	
Number of view fields	840
Repeat distance	100 µm (±5 µm)
Groove width	5 µm (±1 µm)
Groove depth	<5 µm

There are six grids (one in each well) numbered from 1 to 6. The squares are centered in the wells. Each consists of four major squares which are separated in 15 × 15 observation fields and indicated by letters and numbers ranging from:

- A to P (J not used) and 1 to 15
- A to P (J not used) and 16 to 30
- Q to e (capital letters continued with small letters) and 1 to 15
- Q to e (capital letters continued with small letters) and 16 to 30

The grid is made of dots forming grooves, which are 5 µm (±1 µm) wide and approximately 5 µm deep. Cells can grow in the grooves as well. We recommend using objective lenses up to 20×. Anyhow, the optical quality meets the requirements of 63× and 100× oil objective lenses as well (ibidi Standard Bottom, No. 1.5).



Coating your µ–Slide CorrSight™ Live

The uncoated µ–Slide is manufactured from hydrophobic plastic. For the cultivation of most cell lines, it is indispensable to treat the uncoated µ–Slide with biopolymers, which mediate cell adhesion and growth, eg. Collagen IV, Fibronectin, Poly–L–Lysin, and Poly–D–Lysin.

Option 1:

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply 30 µl per well and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with the recommended protein dilution buffer.
- Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Option 2:

- Prepare your coating solution according to the manufacturer's specifications or reference.

- Close the wells with the enclosed polymer coverslip. Therefore remove the protective foil on the slide and on the polymer coverslip and attach the coverslip on the sticky part of the slide.
- Apply 130 µl per channel and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with the recommended protein dilution buffer. You can add the buffer into one channel end and simultaneously aspirate it on the other side.
- Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Detailed information about coatings is provided in Application Note 08 ”Cell culture coating”.

Seeding Cells

Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 0.7-1.7× 10^5 cells/ml suspension should result in a confluent layer within 2-3 days.

Option 1:

- Apply 30 µl cell suspension into each well of the precoated µ–Slide. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover the slide with the supplied lid. Incubate at 37°C and 5% CO_2 as usual.
- Await cell attachment.
- Now close the wells with the enclosed polymer coverslip. Therefore remove the protective foil on the slide and on the polymer coverslip and attach the coverslip on the sticky part of the slide.
- For flow applications fill the channels with 70 µl cell free medium, to flush the air out of the channels. Afterwards fill each reservoir with 60 µl cell free medium.

Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. However, best results might be achieved when the medium is changed every 1-2 days. Carefully aspirate the old medium and replace it by 30 µl/well fresh medium. The volume of a single well is very small. Depending on your cell type the medium might be consumed after some hours. If you want to incubate your cells for longer than a couple of hours we recommend to aspirate and refill cell medium every day.

Tip:

If the wells are properly filled with 30 µl, the liquid surface is planar and in good alignment with the µ–Slides surface. This is how you will be able to observe the whole well area with unimpaired phase contrast.

Tip:

Optionally the wells can be left open for application non-perfusion based applications

Option 2:

- Close the wells with the enclosed polymer coverslip. Therefore remove the protective foil on the slide and on the polymer coverslip and attach the coverslip on the sticky part of the slide.
- Apply 130 µl cell suspension into each channel of the precoated µ–Slide. Be careful to avoid air bubbles.
- Await cell attachment in order not to flush out the cells. Afterwards fill each reservoir with 60 µl cell free medium.

Tip:

The day before seeding the cells we recommend placing the cell medium and the µ–Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time. Trapped air bubbles can be removed from the channel by inclining the µ–Slide and knocking at one edge.

Preparation for Cell Microscopy

To analyze your cells, no special preparations are necessary. Cells can be observed live, or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide 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 of only 180 µm, high resolution microscopy is possible.

Correlative Light and Electron Microscopy (CLEM)

For a detailed CLEM protocol please refer to the FEI website: www.fei.com.

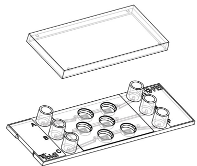
Immersion Oil

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

Ordering Information

The µ–Slide CorrSight™ Live is available with Uncoated surface only.



Cat. No.	Description
80361	**µ–Slide CorrSight™ Live Uncoated**: #1.5 polymer coverslip, hydrophobic, grid repeat distance 100 µm, sterilized

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.

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