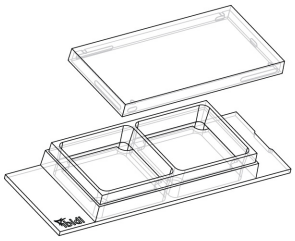


The ibidi product family is comprised of a variety of µ–Slides and µ–Dishes, which have all been designed for high–end microscopic analysis of fixed or living cells.

The glass bottom versions of the µ–Slides and µ–Dishes are especially designed for TIRF and single molecule applications.

The µ–Slide 2 Well Ph+ Glass Bottom (Phase contrast plus) is an array of 2 square fields where cells can be cultivated and investigated with microscopical methods. The µ–Slide 2 Well Ph+ Glass Bottom improves the optical quality of phase contrast microscopy. In contrast to the classic µ–Slide 2 Well, the Ph+ version provides a special plate in the center of the wells. This plate suppresses the meniscus which is disturbing the phase contrast effect in normal open wells. Openings near the corners provide access to the wells for filling and aspirating liquids easily.

Material

The glass bottom version of the µ–Slides are made of a standard µ–Slide but with a glass coverslip bottom. It is not possible to detach the bottom. The µ–Slides are not autoclavable since they are temperature stable only up to 80°C / 175°F.

Optical Properties ibidi Glass Bottom	
Refractive index n_D	1.523
Abbe number	55
Thickness	No. 1.5H (selected quality 170 µm, ± 5 µm)
Material	Schott borosilicate glass, D 263M

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

Geometry

The µ–Slide 2 Well Ph+ Glass Bottom provides a standard slide format according to ISO 8037/1.

Geometry of µ–Slide 2 Well Ph+ Glass Bottom	
Number of wells	2
Dimensions of wells (w × l × h) in mm	21.2 × 23.3 × 3.0
Growth area per well	4.8 cm^2
Coating area per well	11.4 cm^2
Volume per well	1.5 ml
Liquid height	3.0 mm
Total height with lid	10.8 mm
Bottom matches coverslip	No. 1.5

Surface and coating

The µ–Slide 2 Well Ph+ Glass Bottom is manufactured with an uncoated glass coverslip. Washing steps (e.g. with PBS) before cell seeding can remove glass dust which is advantageous for direct cell growth on the surface.

Protein coatings increase direct cell growth of adherent cells. Specific coatings on glass are possible following this protocol:

- Prepare your coating solution according to the manufacturer's specifications or reference. Prepare your µ–Slide. Adjust the concentration to a coating area of 11.4 cm^2 and 1.5 ml.

- Apply 1.5 ml into the growth area. Make sure that the entire bottom is covered with liquid easily tilting or shaking the µ–Slide. Put on the lid 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!

Solvents for Fixation and Staining

Cells can be observed live or fixed directly in the µ–Slide preferably on an inverted microscope. The slide material is compatible to acids, alkalis, PFA, and silicone oil. Alcohols may be used for short term incubation (e.g. cell fixation). Acetone is not compatible. Further specifications can be found at www.ibidi.com.

For optimal results in fluorescence microscopy and storage of stained probes ibidi provides a mounting medium (50001) optimized for µ–Dishes and µ–Slides.

Seeding Cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 5-11 × 10^4 cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 1.5 ml cell suspension into each well of the 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.

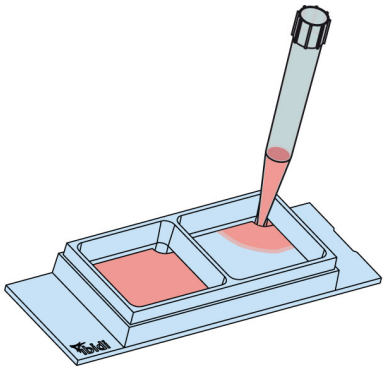
Undemanding cells can be left in their seeding medium for up to three days and grow to confluence there. However, best results might be achieved when the medium is changed every 1–2 days. Carefully aspirate the old medium and replace it by 1.5 ml/well fresh medium.

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 from emerging air bubbles over the incubation time.

Filling and Handling

Fill the wells by using a standard pipet. Inject the cell suspension directly into one of the openings. Medium exchange is easily done by aspirating the entire volume and refilling using 1.5 ml per well.

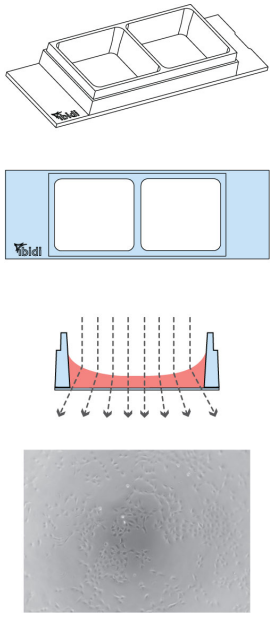
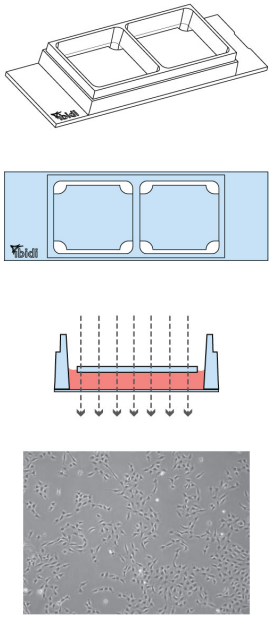


Immersion Oil

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

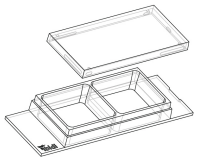
µ–Slide 2 well Ph+ selection guide

µ–Slide 2 Well	µ–Slide 2 Well Ph+
Standard open wells for maximum sample access. Meniscus disturbs the beam path. Good phase contrast quality only in the center of each well	Special plate in the center of the wells suppresses meniscus formation. No meniscus – parallel beam path. For excellent phase contrast microscopy all over the wells.
	

Ordering Information

The µ–Slide 2 Well is available as open well and as a Ph+ version, as well as in a glass bottom version. See table below for choosing your µ–Slide 2 Well.

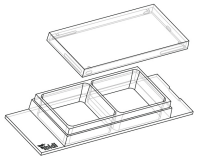
µ–Slide 2 Well



Cat. No.	Description
80886	**µ–Slide 2 Well ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
80882	**µ–Slide 2 Well Collagen IV**: #1.5 polymer coverslip, sterilized
80883	**µ–Slide 2 Well Fibronectin**: #1.5 polymer coverslip, sterilized*
80884	**µ–Slide 2 Well Poly-L-Lysine**: #1.5 polymer coverslip, sterilized
80885	**µ–Slide 2 Well Poly-D-Lysine**: #1.5 polymer coverslip, sterilized*
80881	**µ–Slide 2 Well Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
80887	**µ–Slide 2 Well Glass Bottom**: 1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

* available on request only

µ–Slide 2 Well Ph+



Cat. No.	Description
80296	**µ–Slide 2 Well Ph+ ibiTreat**: #1.5 polymer coverslip, tissue culture treated, sterilized
80292	**µ–Slide 2 Well Ph+ Collagen IV**: #1.5 polymer coverslip, sterilized
80293	**µ–Slide 2 Well Ph+ Fibronectin**: #1.5 polymer coverslip, sterilized*
80294	**µ–Slide 2 Well Ph+ Poly-L-Lysine**: #1.5 polymer coverslip, sterilized
80295	**µ–Slide 2 Well Ph+ Poly-D-Lysine**: #1.5 polymer coverslip, sterilized*
80291	**µ–Slide 2 Well Ph+ Uncoated**: #1.5 polymer coverslip, hydrophobic, sterilized
80297	**µ–Slide 2 Well Ph+ Glass Bottom**: 1.5H (170 µm ±5 µm) D 263 M Schott glass, sterilized

* available on request only

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.