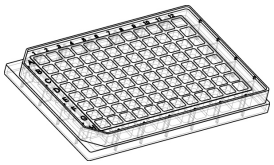
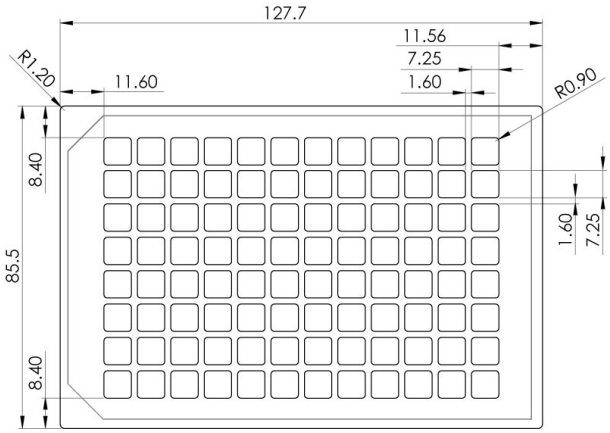


The ibidi product family comprises a variety of different shapes of µ–Slides and µ–Dishes which all have been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.
The µ–Plate 96 well allows you to perform high resolution microscopy in a standard multi–well format.

## Material

The ibidi µ–Plate consists of a plastic with highest optical quality. The bottom material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the µ–Plate. The µ–Plate is not autoclavable since it is temperature stable up to 80°C/175°F only. Please note that gas exchange between the liquid and the incubator's atmosphere occurs partially through the plastic bottom which should not be covered.




## Geometry of the µ–Plate 96 well

The µ–Plate 96 well provides standard geometry and numbering (A-H, 1-12). The bottom of the µ–Plate 96 well provides a high accuracy.

| Dimensions of the µ–Plate 96 well in mm | | |
|---|---|---|
| Length | 127.7 | ± 0.2 |
| Width | 85.5 | ± 0.2 |
| Height with lid | 17.2 | ± 0.4 |
| Height without lid | 15.0 | ± 0.4 |
| Single well depth | 13.0 | ± 0.2 |
| Well to well distance | 9.1 | ± 0.1 |
| Single well dimensions | 7.3 x 7.3 | ± 0.1 |
| **Single well parameters of the µ–Plate 96 well** | | |
| Volume | 300 µl | |
| Growth area | 0.55 $cm^2$ | |
| Coating area using 300 µl | 2.35 $cm^2$ | |
| **Accuracy of the µ–Plate 96 well bottom** | | |
| Inner well flatness | ± 5 µm | |
| Whole plate flatness | ± 25 µm | |
| Bottom matches coverslip | No. 1.5 | |

The µ–Plate 96 well meets all important values of the ANSI/SBS Standards (1-2004, 2-2004, 3-2004 and 4-2004).

## µ–Plate 96 well surface

The µ–Plate 96 well is available with ibiTreat and uncoated surface. The ibiTreat surface is a physical treatment and optimized for adhesion of most cell types. Many cell lines as well as primary cells were tested.
A specific coating of the µ–Plate 96 well can be done yourself following the procedure in section Coating your µ–Plate 96 well.

## Coating your µ–Plate 96 well

The uncoated µ–Plate 96 well must be coated to promote cell adhesion. If you like to establish a certain coating for your demands we recommend to test your coating procedure on uncoated and ibiTreat µ–Plate 96 well, since we have observed that some biomolecules adhere differently to hydrophobic or hydrophilic plastic surfaces.

- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply 300 µl in each well. The coating area using 300 µl is 2.35 $cm^2$.
- Follow your coating protocol.

Further information about coatings are provided in Application Note 08 "Cell culture coating".

## Remove the protection film before usage

The bottom of the µ–Plate is covered with a film to protect the optical quality of the plastic surface. Please pull off the protection film before usage!

## Seeding cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 2–5 × $10^4$ cells/ml suspension should result in a confluent layer within 2–3 days.
- Apply 300 µl cell suspension into each single well. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover the µ–Plate 96 well with the supplied lid. Incubate at 37°C and 5 % $CO_2$ as usual.

**We recommend not to fill more than 600 µl into the µ–Plate 96 well in order to avoid liquid contacting the lid.**

Undemanding cells can be left in their seeding medium for several days and grow to confluence there. However, best results might be achieved when the medium is changed every 2–3 days. Carefully aspirate the old medium and replace it by 300 µl/well fresh medium.

### Tip:

You can stack the µ–Plates 96 well to save space in you incubator. This will not affect cell growth. We recommend making batches with up to 6 plates, due to stability reasons.

## Preparation for cell microscopy

For analyzing your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Plate preferably on an inverted microscope. You can use any fixative of your choice. The µ–Plate 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.

## Immersion oil

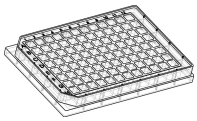
When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the plastic material and the objective.

| Company | Product | Ordering number |
|---|---|---|
| Cargille | type DF, Formula Code: 1261 | (Cargille) 16242 |
| Zeiss | 518 F | (Zeiss) 444960 |
| Olympus | 50CC | (Olympus) 35506 |
| Nikon | 50 CCM DF | (Nikon) MXA 20351 |
| Leica | immersion oil, low fluorescence | (Leica) 11513859 |

## µ–Plate family

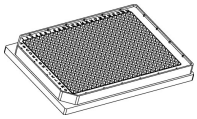
The µ–Plate 96 well and µ–Plate 384 well are available with the following surfaces. Anyhow, please do not hesitate to contact us for other specifications.

µ–Plate 96 well



| Ordering number | Treatment or Coating | Characteristics |
|---|---|---|
| 89626 | ibiTreat, tissue culture treated, sterile | hydrophilic, tissue culture treated, quadratic wells |
| 89621 | uncoated, sterile | hydrophobic, quadratic wells |

µ–Plate 384 well



| Ordering number | Treatment or Coating | Characteristics |
|---|---|---|
| 88401 | uncoated, sterile | hydrophobic, quadratic wells |

## Selected cell tests on different surfaces

Many eukaryotic and bacterial cells have been tested by ibidi on the different surfaces of the µ-Slides. A variety of other cell lines like COS, CHO, HepG2, and NIH 3T3 were successfully grown by our customers.

| | ibiTreat | Collagen IV | Fibronectin | Poly-L-Lysin | Poly-D-Lysin | uncoated |
|---|---|---|---|---|---|---|
| HUVEC | excellent | good | excellent | no cell growth | not done | no cell growth |
| Rat1 | excellent | excellent | excellent | excellent | excellent | poor |
| HT1080 | excellent | excellent | excellent | excellent | not done | poor |
| HeLa | excellent | excellent | excellent | excellent | not done | poor |
| Neuro2A | excellent | excellent | excellent | excellent | excellent | poor |
| PC12 | good | excellent | excellent | excellent | excellent | no cell growth |
| *Dictyostelium discoideum* | not done | excellent | not done | not done | not done | excellent |
| *Escherichia coli* | excellent | not done | not done | excellent | not done | excellent |

HUVEC = Human Umbilical Vein Endothelial Cells
Rat1 = Rat Fibroblast
HT1080 = Human Fibrosarcoma
HeLa = Human Cervix Adenocarcinoma
Neuro2A = Mouse Neuroblastoma
PC12 = Rat Pheochromocytom

*Dictyostelium discoideum* = strain wild type AX-2

*Escherichia coli* = strain MDG131

## 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.