The µ–Chamber 12 well is a removable silicone chamber for cell culture and immunofluorescence stainings. It allows the use of standard cultivation, staining and mounting techniques with coverslip sealing. µ–Chamber 12 well supports upright microscopy and long-term storage of cell culture microscopy samples after mounting the glass slide with a coverslip[1]. µ–Chamber 12 well is not recommended for live cell microscopy on inverted microscopes since cells grow on a 1 mm microscopy glass slide.

[1] Suitable 24 mm × 60 mm coverslips are provided by ibidi (10811).

Material

The µ–Chamber 12 well is comprised of a self–adhesive 12 well silicone gasket mounted on a standard microscopy glass slide. The gasket is manufactured from biocompatible silicone material. Although both materials are autoclavable and compatible with alcohols, we do not recommend reusing them.

Geometry

µ–Chamber 12 well provides a standard slide format according to ISO 8037/1.

Geometry of the µ–Chamber 12 well	
Number of wells	12
Dimension of wells (w × l × h) in mm	7.5 × 7.5 × 8
Volume per well	250 µl
Total height with lid	11 mm
Growth area per well	0.56 cm^2
Coating area per well	1.9 cm^2
Bottom material, size in mm	standard glass slide 26 × 76 × 1, ground edges, twin frosted ends 13 mm

Surfaces and Coatings

µ–Chamber 12 well is mounted on an uncoated glass slide with twin frosted ends. Specific coatings on glass are possible following this protocol:

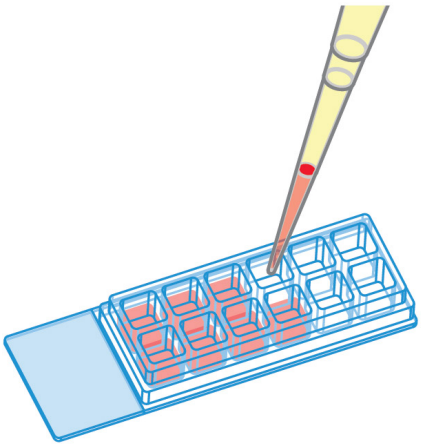
- Prepare your coating solution according to the manufacturer's specifications or reference.
- Apply 250 µl per well and leave at room temperature for at least 30 minutes.
- Aspirate the solution and wash with ultra-pure water. Let dry at room temperature.

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 × 10^4 cells/ml suspension should result in a confluent layer within 2 - 3 days.

- Apply 250 µl cell suspension into each well. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- Cover wells 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 250 µl/well fresh medium.



Solvents for Fixation, Staining and Other Purposes

µ–Chamber 12 well is compatible to methanol, acetone, acids, alkalis, PFA, DMSO, silicone oil, and mineral oil for cell culture.

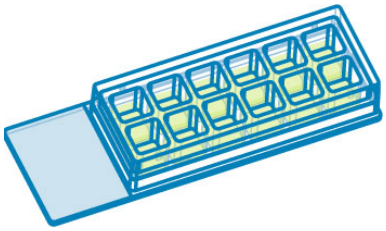
Immunofluorescence Microscopy

After cultivation, cells can be fixed and stained before or after removing the silicone gasket.

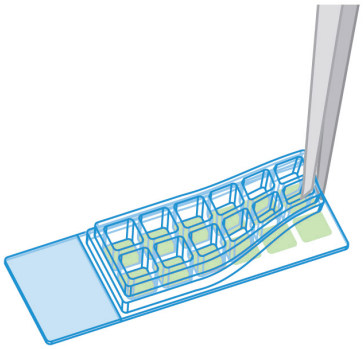
Single well technique:

- Carry out all necessary steps (fixation, permeabilization, staining, washing) in single wells (1).
- Starting from one edge, remove the silicone gasket by hand or tweezers (2).
- Mount slide with a permanent mounting medium of your choice and a 24 mm × 60 mm coverslip (10811) (3).

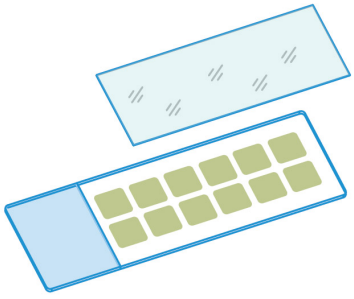
(1)



(2)



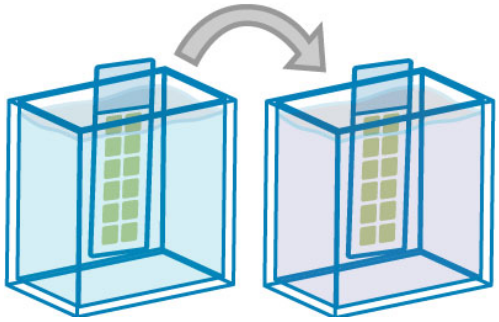
(3)



Parallel technique:

- Starting from one edge, remove the silicone gasket by hand or tweezers (2).
- Carry out all necessary steps (fixation, permeabilization, staining, washing) dipping the slide into the solutions. A coverslip (24 mm × 60 mm) can be used during staining for reducing the volume (4).
- Mount slide and coverslip with a permanent mounting medium of your choice and a coverslip 24 mm × 60 mm (10811) (3).

(4)



ibidi Mounting Medium is not recommended for µ–Chamber 12 well because it is non–hardening and stays a liquid (which is advantageous for µ–Slides and µ–Dishes).

Tips:

The day before seeding the cells we recommend placing the cell medium and slides into the incubator for equilibration. This will prevent the liquid inside the slide or channel from creating air bubbles over the incubation time.
For mounting of slide samples, a permanent mounting medium is recommended. ibidi Mounting Medium is not recommended because it is non–hardening and stays a liquid (which is advantageous for µ–Slides and µ–Dishes).

µ–Chamber 12 well Family

Ordering Number	Product Name	Characteristics
81201	µ–Chamber 12 well	Standard glass slide
10811	glass coverslips, unsterile	24 mm × 60 mm, No. 1.5 (selected, 170 ± 10 µm)

Selected References

R. I. Dmitriev, H. Ropiak, G. Ponomarev, D. V. Yashunsky, and D. B. Papkovsky. Cell-Penetrating Conjugates of Coproporphyrins with Oligoarginine Peptides: Rational Design and Application for Sensing Intracellular O2. *Bioconjugate Chemistry*, 2011. doi: 10.1021/bc200324q.

K. Koren, R. I. Dmitriev, S. M. Borisov, D. B. Papkovsky, and I. Klimant. Complexes of IrIII-Octaethylporphyrin with Peptides as Probes for Sensing Cellular O2. *ChemBioChem*, 2012. doi: 10.1002/cbic.201200083.

J. Mikeš, M. Hýžďalová, L. Kočí, R. Jendželovský, J. Kovaľ, A. Vaculová, J. Hofmanová, A. Kozubík, and P. Fedoročko. Lower sensitivity of FHC fetal colon epithelial cells to photodynamic therapy compared to HT-29 colon adenocarcinoma cells despite higher intracellular accumulation of hypericin. *Photochem. Photobiol. Sci.*, 2011. doi: 10.1039/C0PP00359J.

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