

The 3 Well Chamber, removable is a removable silicone chamber, mounted on a glass slide, for cell culture and immunofluorescence stainings. It allows the use of standard cultivation, staining and mounting techniques with coverslip sealing.

After mounting the glass slide with a coverslip, it is ready for the use with upright and inverted microscopes, as well as for long term storage. Suitable 24 mm×60 mm coverslips are provided by ibidi (10811).

The special geometry of the 3 Well Chamber, removable provides a structure inside each well, forming a round inner well. The option of placing a round coverslip (ibidi, 10815) into the well allows the user to fill the inner well exclusively, by minimizing the volume and eliminating the meniscus.

Material

The 3 Well Chamber, removable is comprised of a self-adhesive silicone gasket mounted on a standard microscopy glass slide. The gasket is manufactured from bio-compatible silicone material. Although both materials are autoclavable and compatible with alcohols, we do not recommend reusing them. The glass bottom exhibits ground edges and twin frosted ends.

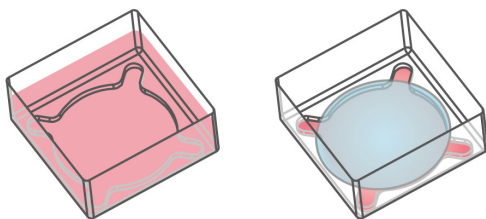
Geometry

The 3 Well Chamber, removable provides a standard slide format according to ISO 8037/1.

General Dimensions

Number of wells	3
Bottom size (w × l × h)	26 × 76 × 1 mm
Twin frosted ends	13 mm
Total height with lid	11 mm

The single wells of the 3 Well Chamber, removable consist of the outer rectangular well with an inlet forming a round inner well. When placing a coverslip with 15 mm diameter onto the inlet, a separate chamber is locked up. This assures a homogeneous distribution of cells or substances and minimizes the liquid volume. The separate chamber can be accessed via four inlets in the edges.



Left: Single well filled with 1.1 ml medium. Right: Volume reduction by use of the coverslip (150 µl medium).

Dimensions of the Single Wells

Dimension of wells (w × l × h)	16.5 × 16.5 × 8 mm
Volume per well	1100 µl
Reduced volume per well	150 µl
Growth area per well	1.66 cm ²
Coating area per well	3.37 cm ²
Diameter of inner well	13.5 mm
Height of the inner well	0.75 mm

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-L-Lysine	18 months

Surface and Coating

The 3 Well Chamber, removable is mounted on an uncoated glass slide with twin frosted ends. 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 slide. Adjust the concentration to a coating area of 3.37 cm² and a coating volume of 1100 µl per well.
- Apply 1100 µl into each well. Make sure that the entire bottom is covered with liquid by slightly tilting or shaking the slide. Put on the lid and leave the slide 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!

Seeding Cells

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a $2-6 \times 10^4$ cells/ml suspension should result in a confluent layer within 2-3 days.
- Apply 1100 µl cell suspension into each well of the 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% CO₂ 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 1100 µl fresh medium per well.

Note:

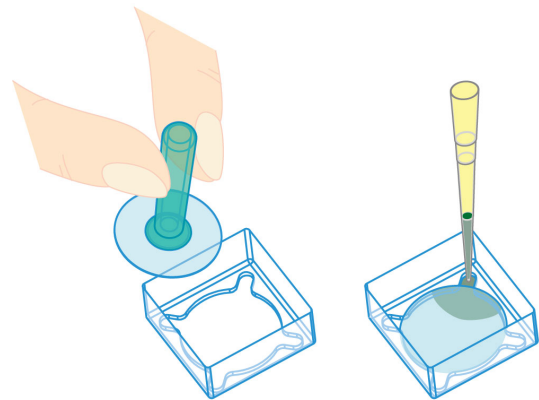
For a more homogeneous cell distribution use the 15 mm coverslips (ibidi, 10815). Autoclave the coverslips prior to use. Please follow the protocol below. Use 150 µl of cell suspension with a concentration of $1.7-3.8 \times 10^5$ cells/ml.

Handling of the Round Coverslip

For filling the chamber by use of the round coverslip, follow this protocol. One Coverslip Pick-Up Tool is delivered with every unit of 15 mm coverslips (ibidi, 10815):

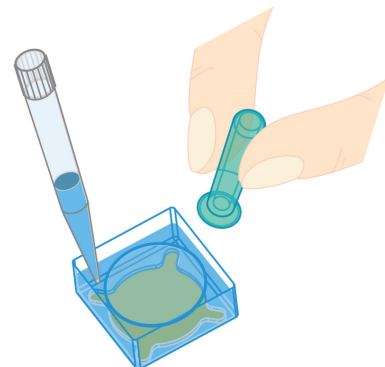
- Before applying the coverslip, the well must be emptied.

- Grab the coverslip with the Pick-Up Tool and place it onto the well inlet. The Pick-Up Tool works like a Pasteur pipette.
- Inject a volume of 150 µl into one of the edges, that are kept open.



Remove the coverslip as follows:

- Add 950 µl in one corner next to the coverslip.
- Grab the floating coverslip with the Pick-Up Tool or tweezers and remove it from the well.



Solvents for Fixation, Staining and Other Purposes

3 Well Chamber, removable is compatible to methanol, acetone, acids, alkalis, PFA, DMSO, silicone oil, and mineral oil for cell culture.

Immunofluorescence Microscopy

After cultivation, the cells can be fixed and stained in two different ways.

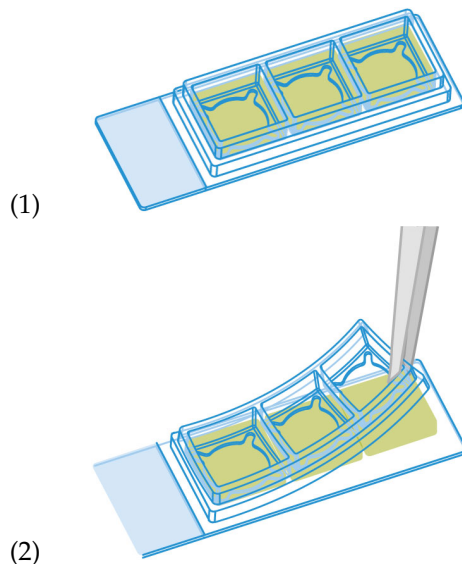
Single well technique:

All steps are carried out in the single wells before removing the silicone gasket. Using the 15 mm coverslips will reduce the volume of the staining solution to 150 µl.

Instructions

3 Well Chamber, removable

- Perform your standard staining protocol (fixation, permeabilization, staining, washing) in the single wells (1).
- Starting from one edge, remove the silicone gasket by hand or by using tweezers (2).



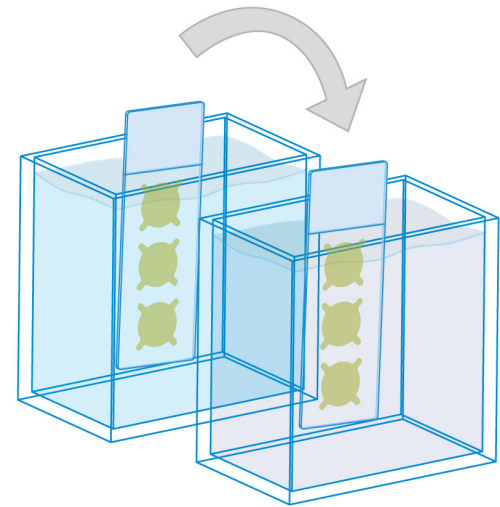
Note:

If you want to reduce the volume of the staining solution needed, use the round coverslip as shown in the coverslip handling protocol. Wash the well by adding 950 μ l and removing the coverslip.

Parallel technique:

All necessary steps (fixation, permeabilization, staining, washing) are carried out by dipping the whole slide into the solutions after removing the silicone gasket (4).

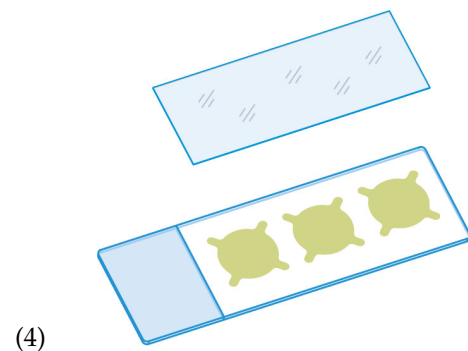
- Starting from one edge, remove the silicone gasket by hand or by using tweezers (2).
- Perform your standard staining protocol (fixation, permeabilization, staining, washing) by dipping the slide into the solutions (3).



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Mounting:

Mount the slide with a coverslip 24 mm \times 60 mm (ibidi, 10811) and a permanent mounting medium of your choice (4).



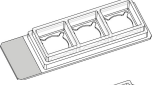
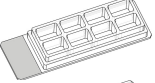
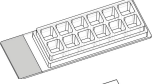

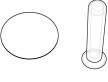
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Tip:

For mounting of slide samples, a hardening permanent mounting medium such as Fluoroshield™ (Sigma-Aldrich), Vectashield® (Vector Laboratories Inc.) or ProLong® Antifade (ThermoFisher Scientific) 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).

Ordering Information

	Cat. No.	Description
	80381	3 Well Chamber, removable: microscopy glass slide, sterilized
	80841	8 Well Chamber, removable: microscopy glass slide, sterilized
	81201	12 Well Chamber, removable: microscopy glass slide, sterilized
	10811	Coverslips for Chambers, removable, # 1.5H ($170 \pm 5 \mu\text{m}$) D263 M Schott glass, 24 mm × 60 mm, unsterile
	10815	Coverslips and Coverslip Pick-Up Tool for 3 Well Chamber, removable: # 1.5H ($170 \pm 5 \mu\text{m}$) D263 M Schott glass, \varnothing 15 mm, unsterile, including one silicone rubber tool for convenient glass coverslip handling

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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