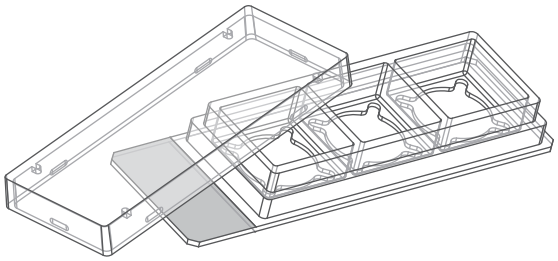
# 3 Well Chamber, removable

Instruction Manual



The 3 Well Chamber, removable is a silicone chamber mounted on a glass slide, designed for cell culture and immunofluorescence (IF) staining. The removable chamber allows standard cultivation, staining, and mounting procedures using coverslip sealing. After removing the chamber and mounting the glass slide with a coverslip, the sample can be analyzed using upright or inverted microscopes and is suitable for long-term storage. Compatible 24 × 60 mm coverslips are available from ibidi (#10811).
The special design of the 3 Well Chamber, removable creates a round inner well within each well. Placing a round coverslip (ibidi, 10815) into the well allows filling only the inner well, minimizing the required volume and eliminating meniscus formation.

This document applies to the following product:

| | |
|---|---|
| 80381 | **3 Well Chamber, removable** |

## Material

The 3 Well Chamber, removable consists of a self-adhesive silicone gasket mounted on a standard microscopy glass slide. The gasket is made of biocompatible silicone. Although both components are autoclavable and compatible with alcohols, reuse is not recommended. The glass slide features ground edges and a frosted labeling area.

**CAUTION** – Be cautious when handling ibidi labware products with a glass bottom! The glass coverslip or slide is fragile and can break easily. Handle these items carefully to prevent physical injury and damage to devices due to medium leakage.

## Surface

The 3 Well Chamber, removable is a removable silicone chamber, mounted on an untreated standard microscopy glass slide. Washing steps (e.g., with PBS) before cell seeding helps remove glass dust, which enhances direct cell growth on the surface.

## Shipping and Storage

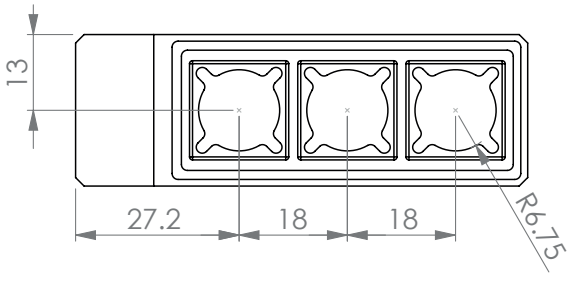
This product is sterilized and sealed in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is outlined in the following table.

| Conditions | |
|---|---|
| Shipping conditions | Ambient |
| Storage conditions | RT (15–25 °C) |

| Shelf Life | |
|---|---|
| Removable silicone chamber | 36 months |

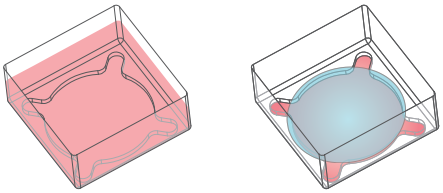
## Geometry

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



| Specifications | |
|---|---|
| Number of wells | 3 |
| Bottom size (w × l × h) | 26 × 76 × 1 mm |
| Height with lid | 11 mm |
| Dimensions of wells (w × l × h) | 16.5 × 16.5 × 8 mm |
| Diameter of inner well | 13.5 mm |
| Height of the inner well | 0.75 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 $cm^2$ |

Each well of the 3 Well Chamber, removable consists of an outer rectangular well with a central inlet that forms a round inner well. When a round 15 mm coverslip is placed onto the inlet, a separate chamber is created. This ensures homogeneous distribution of cells or substances and minimizes the required liquid volume. The chamber can be accessed through four inlets located at the edges.

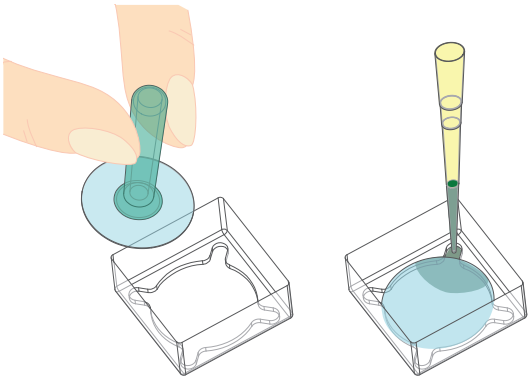


*Left: Single well filled with 1.1 ml medium. Right: Reduced volume by use of the coverslip (150 µl of medium).*

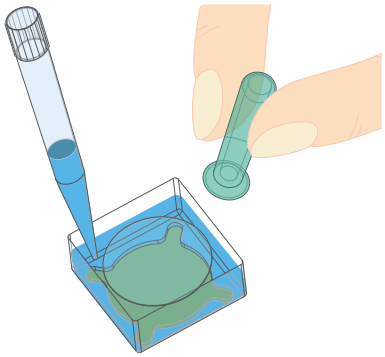
## Handling of the Round Coverslip

To fill the chamber with the round coverslip in place, follow the protocol below.

1. Before placing the coverslip, the well must be emptied.
2. Pick up the coverslip with the Pick-Up Tool and place it onto the well inlet. The Pick-Up Tool works similarly to a Pasteur pipette. One Coverslip Pick-Up Tool is included with every unit of 15 mm coverslips (ibidi, 10815)
3. Inject 150 µl of solution through one of the open edges.



4. To wash the well and remove the coverslip, add 950 µl of solution through one of the open edges.
5. Once the coverslip floats up, grab it with the Pick-Up Tool or tweezers and remove it from the well.



## Coating

Detailed information about coatings is provided in Application Note 08: Coating Protocols for ibidi Labware.

In short, specific coatings are possible following this protocol:

1. Prepare your coating solution according to the manufacturer's specifications. Adjust the concentration to a coating area of 3.37 $cm^2$ and a volume of 1100 µl per well.
2. Apply 1100 µl coating solution per well. Make sure that the entire bottom is covered with liquid by slightly tilting or shaking the slide. Put on the lid and leave it at room temperature for at least 30 minutes.

3. Aspirate the solution and wash with the recommended protein dilution buffer.
4. The coated slide is ready to be used. Be aware that allowing the coated surface to dry out is not recommended, as some coating proteins may degrade upon drying.

## Seeding Cells

1. Trypsinize and count the cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of a 2–6 × $10^4$ cells/ml suspension should result in a confluent layer within 2–3 days.
2. Apply 1100 µl cell suspension per well. Avoid shaking, as this will result in inhomogeneous cell distribution.
3. Cover the slide with the supplied lid. Incubate as usual (e.g., at 37 °C and 5% $CO_2$).

Insensitive cells can be left in their seeding medium for several days and grow to confluence there. However, optimal results might be achieved when the medium is changed every 2–3 days. For this, carefully aspirate the old medium and replace it by 1100 µl fresh medium per well.

**TIP** – For a more homogeneous cell distribution, use the 15 mm coverslips (ibidi, 10815). Autoclave the coverslips prior to use. Use 150 µl of cell suspension with a concentration of 1.7–3.8 × $10^5$ cells/ml.

**NOTE** – The 3 Well Chamber, removable is not recommended for high-resolution live cell imaging on inverted microscopes, as cells grow on a 1 mm thick microscopy glass slide.

## IF Handling and Processing

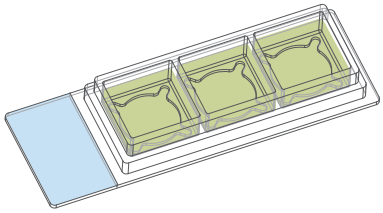
After cultivation, cells can be fixed and stained using one of the following two approaches. The slide is then mounted for microscopy.

### a) Single-Well Staining

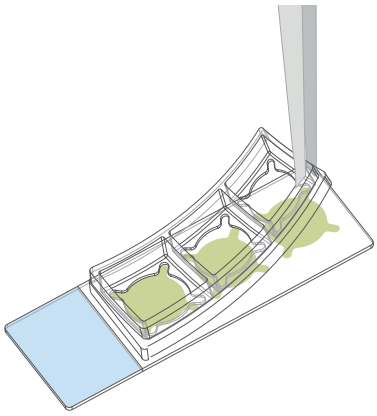
All steps (fixation, permeabilization, staining, and washing) are performed within individual wells before removing the silicone gasket.

**NOTE** – To reduce the volume of required staining solution, use the round coverslip as described in the Handling of the Round Coverslip.

1. Perform your standard staining protocol directly in the individual wells.



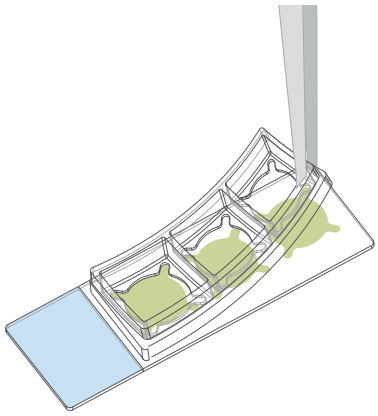
2. Remove the silicone gasket by carefully lifting one edge with tweezers or by hand.



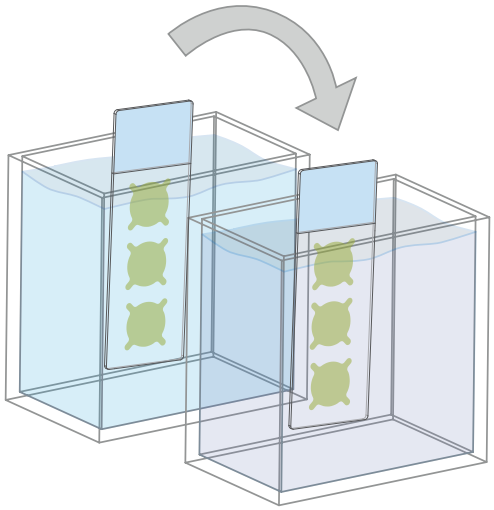
### b) Parallel Staining

All steps (fixation, permeabilization, staining, and washing) are performed by dipping the entire slide in the respective solutions after removing the silicone gasket.

1. Remove the silicone gasket by carefully lifting one edge with tweezers or by hand.

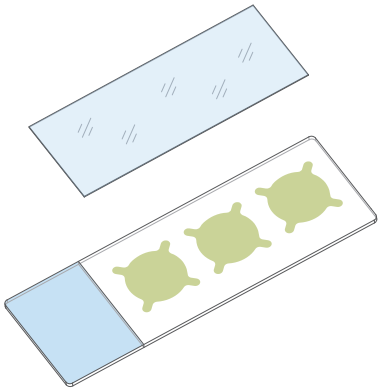


2. Perform your standard staining protocol by sequentially dipping the slide in the respective solutions.



### Mounting

Mount the slide using a 24 × 60 mm coverslip (ibidi #10811) and a permanent mounting medium of your choice.



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

ibidi Mounting Medium is not recommended, as it is non-hardening and remains liquid, which is advantageous for µ-Slides and µ-Dishes but not suitable for mounting slide samples.

## Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the 3 Well Chamber, removable. For a full list of compatible solvents and more information on chemical compatibility, visit ibidi.com/chemicals.

| Chemical / Solvent | Compatibility |
|---|---|
| Methanol | Yes |
| Ethanol | Yes |
| Formaldehyde | Yes |
| Acetone | Yes, without lid |
| Immersion oil | Yes |

**For research use only!**

Further information can be found at ibidi.com. For questions and suggestions, please contact us by e-mail at info@ibidi.com or by telephone at +49 (0)89/520 4617 0.