The ibidi Culture–Insert family is mainly developed for wound healing assays. A special sticky and biocompatible surface at the bottom side works like a glue and avoids leaking. A cell suspension can be placed in the wells allowing to grow cells in the designated areas only. After cell attachment the Culture–Insert can be removed by using sterile tweezers. There are no remains on the surface. The attached cells grow on the designated areas. The Culture–Inserts can be placed on every flat, clean, and dry surface.

The Culture–Insert 2 Well consists of two wells, that are separated by a wall of 500 µm. When both wells are filled with adherent cells, a cell–free gap of approx. 500 µm is created after removing the Culture–Insert 2 Well. The Culture–Insert 2 Well is also intended for co-cultivation and migration studies. Several other applications are possible.

Material

The ibidi Culture–Inserts are manufactured from biocompatible silicone material. Although, the material is autoclavable and compatible to alcohols we do not recommend reusing it.

Please note! When using a ibidi µ–Dish, µ–Slide or µ–Plate take care that the ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 2.

Geometry

The Culture–Insert 2 Well consists of two chambers with the following dimensions:

Dimensions of the Culture–Insert 2 Well	
Number of wells	2
Outer dimensions (w×l×h)	8.4 mm × 8.4 mm × 5 mm
Growth area per well	0.22 cm^2
Coating area per well	0.82 cm^2
Volume per well	70 µl
Width of cell–free gap	500 µm ± 50 µm

The Culture–Insert 2 Well 24 is based on the ibidi µ–Plate 24 Well. The dimensions of the µ–Plate are listed in the table below.

Dimensions of the Culture–Insert 2 Well 24	
Well to well distance	18.7 ±0.1 mm
Culture–Insert 2 Well position accuracy	±0.3 mm

More detailed information is provided in the instructions of the µ–Plate 24 Well.

We recommend using the Culture–Insert 2 Well in ibidi µ–Dishes, µ–Slide 2 Well, µ–Slide 4 Well or µ–Plate 24 Well.

The Culture–Insert 2 Well will also fit in standard 6 Well plates, 12 Well plates or petri dishes. It is also possible to use them on sterile glass coverslips or glass slides.

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

Surfaces and Coatings

We recommend using the Culture–Insert 2 Well on non-coated (tissue culture treated) surfaces to ensure reproducibility of cell behavior.

Please test the compatibility with your specific protein coating with a free sample available on www.ibidi.com.

The Culture–Insert 2 Well can be transferred to any flat, clean, and dry surface. Use sterile tweezers for transfer and gently push with a finger tip (wear gloves and sterilize with ethanol). Keep in mind that only the bottom side is sticky. Turn around and make sure the bottom is sealed appropriately. Push gently if necessary.

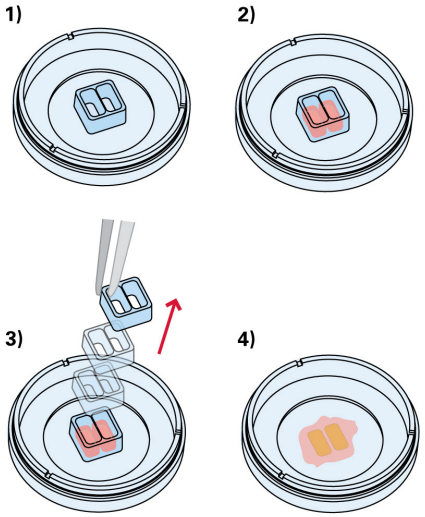
The Culture–Insert 2 Well is not working on wet or moist surfaces. It might also not work on uneven or dusty substrates.

Seeding Cells

For performing a wound healing assay with the ibidi Culture–Insert 2 Well follow the indicated steps. More detailed information is provided in Application Note 21 "Wound Healing Assay" and Application Note 30 "Data Analysis of Wound–Healing Assays".

Wound healing assays using the ibidi Culture–Insert 2 Well are not 100 % comparable to the common scratch assay technique. Since the cell-free gap is created in another way and the surface is different there might be differences to former experimental data.

- Prepare your cell suspension as usual. It is recommended to include a centrifugation step to remove dead cells and cell debris. Depending on your cell type, application of a $3 - 7 \times 10^5$ cells/ml should result in a confluent layer within 24 hours.
- Apply 70 µl into each well. Avoid shaking as this will result in inhomogeneous cell distribution.
- Incubate at 37°C and 5 % CO_2 as usual.
- Optionally, it is possible to fill the outer area with cell suspension or cell culture medium. Use the recommended volume of the dish minus 200 µl.
- After appropriate cell attachment (24 hours) gently remove the Culture–Insert 2 Well by using sterile tweezers. Grab a corner of the Culture–Insert 2 Well.
- Fill the used well or dish with cell free medium. Use the recommended volume (e.g. for µ–Dish $^{35mm, high}$ use 2 ml).
- If necessary, a washing step can help removing non–adherent cells or cell debris.
- Conduct your experiment.



Tip:

In case the cell layer is (partially) detached when removing the Culture–Insert 2 Well, use a smaller seeding density to create a less confluent cell layer or decrease incubation time.

Immersion Oil

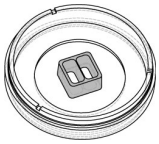
When using oil immersion objectives, use only the immersion oils specified in the table. The use of a non–recommended oil could lead to the damage of the plastic material and the objective.

Company	Product	Ordering Number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

Ordering Information

The Culture–Insert is available with different numbers of wells and in various product versions.

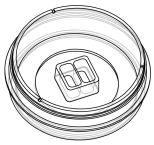
Culture–Insert in µ–Dish $^{35mm, low}$

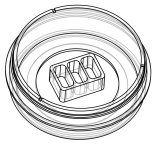


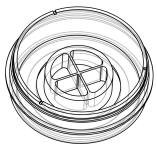
Cat. No.	Description
80206	**Culture–Insert 2 Well in µ–Dish $^{35mm, low}$, ibiTreat**: ready to use, tissue culture treated, sterilized

Culture–Insert in µ–Dish $^{35mm, high}$

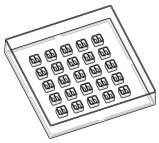
Cat. No.	Description
81176	**Culture–Insert 2 Well in µ–Dish $^{35mm, high}$, ibiTreat**: ready to use, tissue culture treated, sterilized
80366	**Culture–Insert 3 Well in µ–Dish $^{35mm, high}$, ibiTreat**: ready to use, tissue culture treated, sterilized
80466	**Culture–Insert 4 Well in µ–Dish $^{35mm, high}$, ibiTreat**: ready to use, tissue culture treated, sterilized





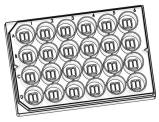


25 Culture–Inserts for self–insertion



Cat. No.	Description
80209	**25 Culture–Inserts 2 Well for self insertion**: in a 10 cm transport dish, sterilized
80369	**25 Culture–Inserts 3 Well for self insertion**: in a 10 cm transport dish, sterilized
80469	**25 Culture–Inserts 4 Well for self insertion**: in a 10 cm transport dish, sterilized

Culture–Insert 24



Cat. No.	Description
80241	**Culture–Insert 2 Well 24, ibiTreat**: a µ–Plate 24 Well with 24 ready to use Culture–Inserts 2 Well, tissue culture treated, sterilized

Selected References

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