

The ibidi Culture-Insert family is mainly developed for wound healing assays. A special sticky and bio-compatible 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 4 Well consists of four wells, representing the four quarters of the round Culture-Insert. The wells are separated by a wall of 500 μm . When the wells are filled with adherent cells, a cell-free gap of 500 μm is created between the adjacent wells after removing the Culture-Insert 4 Well. Additionally, a center gap of 1000 μm is created, allowing to observe the migration of all cell types at once. The Culture-Insert 4 Well is also intended for co-cultivation and migration studies. Several other applications are possible.

Material

The Culture-Insert 4 Well is manufactured from biocompatible silicone. Although, the material is autoclavable and compatible to alcohols, reuse is not recommend.

Please note! When using an ibidi μ -Dish, μ -Slide or μ -Plate, make sure that the ibidi Polymer Coverslip is compatible with the immersion oil you intend to use. See page 2 for the list of compatible oils.

Geometry

The Culture-Insert 4 Well consists of four chambers with the following dimensions:

Dimensions of the Culture-Insert 4 Well	
Number of wells	4
Outer diameter	17 mm
Growth area per well	0.35 cm^2
Coating area per well	1.23 cm^2
Volume per well	110 μl
Width of cell-free gap	
two cell fronts	500 $\mu\text{m} \pm 100 \mu\text{m}$
center span	1000 $\mu\text{m} \pm 100 \mu\text{m}$

We recommend using the Culture-Insert 4 Well in ibidi μ -Dishes, or μ -Slide 2 Well. The Culture-Insert 4 Well will also fit 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	
ibiTreat, Uncoated	36 months

Surfaces and Coatings

We recommend using the Culture-Insert 4 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 4 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 4 Well is not working on wet or moist surfaces. It might also not work on uneven or dusty substrates.

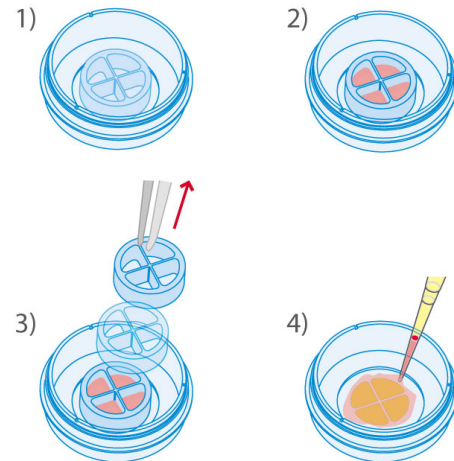
Seeding Cells

For performing a wound healing assay with the ibidi Culture-Insert 4 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 4 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 110 μ l into each well. Avoid shaking as this will result in inhomogeneous cell distribution.
- Incubate at 37°C and 5% CO₂ 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 700 μ l.
- After appropriate cell attachment (24 hours) gently remove the Culture-Insert 4 Well by using sterile tweezers. Grab a corner of the Culture-Insert 4 Well.
- Fill the used well or dish with cell free medium. Use the recommended volume (e.g. for μ -Dish^{35 mm, 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 4 Well, use a smaller seeding density to create a less confluent cell layer or decrease incubation time.

Immersion Oil

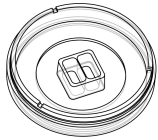
When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non-recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are not listed in the table below should be considered as non-compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil	50101	16-12-27	01/2017
Zeiss	Immersol 518 F	444960	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012
Leica	Immersion Liquid	11513859	n.a.	03/2011
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017

Ordering Information

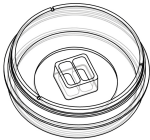
The Culture-Insert is available with different numbers of wells and in various product versions.

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

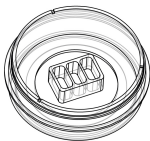


Cat. No.	Description
80206	Culture-Insert 2 Well in μ-Dish^{35 mm, low} ibiTreat: ready to use, tissue culture treated, sterilized

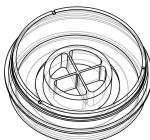
Culture-Insert in μ -Dish^{35 mm, high}



Cat. No.	Description
81176	Culture-Insert 2 Well in μ-Dish^{35 mm, high} ibiTreat: ready to use, tissue culture treated, sterilized

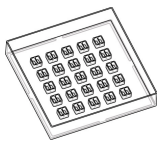


80366	Culture-Insert 3 Well in μ-Dish^{35 mm, high} ibiTreat: ready to use, tissue culture treated, sterilized
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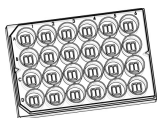
80466	Culture-Insert 4 Well in μ-Dish^{35 mm, high} ibiTreat: ready to use, tissue culture treated, sterilized
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25 Culture-Inserts for self-insertion



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 Black with 24 ready to use Culture-Inserts 2 Well, tissue culture treated, sterilized

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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