



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 3 Well consists of three wells, placed side by side and 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 3 Well. The Culture-Insert 3 Well is also intended for co-cultivation and migration studies. Several other applications are possible.

## Material

The Culture-Insert 3 Well is manufactured from biocompatible silicone. Although, the material is autoclavable and compatible to alcohols, it is intended for one-time use only.

**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 3 Well consists of three chambers with the following dimensions:

Dimensions of the Culture-Insert 3 Well	
Number of wells	3
Outer dimensions (w × l × h)	8.4 mm × 12.15 mm × 5 mm
Growth area per well	0.22 cm <sup>2</sup>
Coating area per well	0.82 cm <sup>2</sup>
Volume per well	70 µl
Width of cell-free gap	500 µm ± 100 µm

We recommend using the Culture-Insert 3 Well in ibidi µ-Dishes, or µ-Slide 2 Well. The Culture-Insert 3 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	
ibiTreat	36 months

## Surfaces and Coatings

We recommend using the Culture-Insert 3 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](http://www.ibidi.com).

The Culture-Insert 3 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 3 Well is not working on wet or moist surfaces. It might also not work on uneven or dusty substrates.

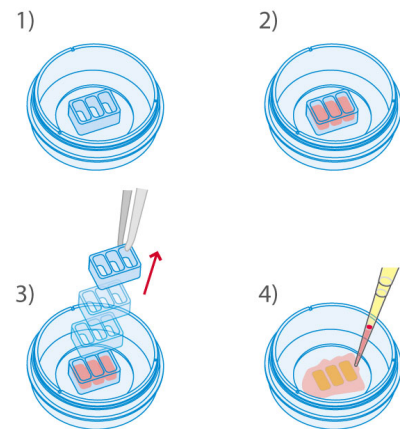
## Seeding Cells

For performing a wound healing assay with the ibidi Culture-Insert 3 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 3 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  $\mu$ l into each well. Avoid shaking as this will result in inhomogeneous cell distribution.
- Incubate at 37°C and 5% CO<sub>2</sub> 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 300  $\mu$ l.
- After appropriate cell attachment (24 hours) gently remove the Culture-Insert 3 Well by using sterile tweezers. Grab a corner of the Culture-Insert 3 Well.
- Fill the used well or dish with cell free medium. Use the recommended volume (e.g. for  $\mu$ -Dish<sup>35 mm, high</sup> 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 3 Well, use a smaller seeding density to create a less confluent cell layer or decrease incubation time.

**Immersion Oil**

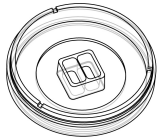
When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non-recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are 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
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017
Leica	Immersion Liquid	11513859	n.a.	03/2011
Nikon	Immersion Oil F2 30cc	MXA22192	n.a.	01/2020
Nikon	Silicone Immersion Oil 30cc	MXA22179	20191101	01/2020
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Zeiss	Immersionol 518 F	444960	160706	01/2017
Zeiss	Immersionol W 2010	444969	101122	04/2012

Ordering Information

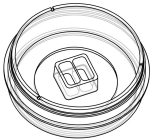
The Culture-Insert is available with different numbers of wells and in various product versions.

Culture-Insert in  $\mu$ -Dish<sup>35 mm, low</sup>

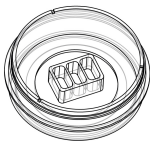


Cat. No.	Description
80206	<b>Culture-Insert 2 Well in <math>\mu</math>-Dish<sup>35 mm, low</sup> ibiTreat:</b> ready to use, tissue culture treated, sterilized

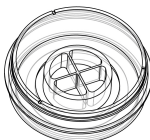
Culture-Insert in  $\mu$ -Dish<sup>35 mm, high</sup>



Cat. No.	Description
81176	<b>Culture-Insert 2 Well in <math>\mu</math>-Dish<sup>35 mm, high</sup> ibiTreat:</b> ready to use, tissue culture treated, sterilized

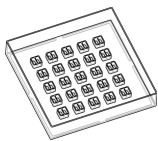


80366	<b>Culture-Insert 3 Well in <math>\mu</math>-Dish<sup>35 mm, high</sup> ibiTreat:</b> ready to use, tissue culture treated, sterilized
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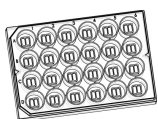
80466	<b>Culture-Insert 4 Well in <math>\mu</math>-Dish<sup>35 mm, high</sup> ibiTreat:</b> ready to use, tissue culture treated, sterilized
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25 Culture-Inserts for self-insertion



Cat. No.	Description
80209	<b>25 Culture-Inserts 2 Well for self-insertion:</b> in a 10 cm transport dish, sterilized
80209-150	<b>25 Culture-Inserts 2 Well for self-insertion, Bulk Pack:</b> in a 10 cm transport dish, sterilized
80369	<b>25 Culture-Inserts 3 Well for self-insertion:</b> in a 10 cm transport dish, sterilized
80469	<b>25 Culture-Inserts 4 Well for self-insertion:</b> in a 10 cm transport dish, sterilized

Culture-Insert 24



Cat. No.	Description
80241	<b>Culture-Insert 2 Well 24 ibiTreat:</b> a $\mu$ -Plate 24 Well Black with 24 ready to use Culture-Inserts 2 Well, tissue culture treated, sterilized

**For research use only!**

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

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