

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  $\mu\text{m}$ . When the wells are filled with adherent cells, a cell-free gap of approx. 500  $\mu\text{m}$  is created between the adjacent wells after removing the Culture-Insert 4 Well. Additionally, a center gap of approximately 1000  $\mu\text{m}$  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 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  $\mu$ -Dish,  $\mu$ -Slide or  $\mu$ -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 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 $\text{cm}^2$
Coating area per well	1.23 $\text{cm}^2$
Volume per well	110 $\mu\text{l}$
Width of cell-free gap	
two cell fronts	500 $\mu\text{m} \pm 50 \mu\text{m}$
center span	1000 $\mu\text{m} \pm 100 \mu\text{m}$

We recommend using the Culture-Insert 4 Well in ibidi  $\mu$ -Dishes, or  $\mu$ -Slide 2Well. 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  $\mu$ -Slides,  $\mu$ -Dishes and  $\mu$ -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 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](http://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.

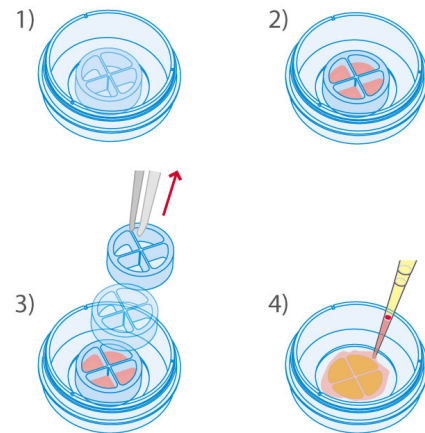
The Culture-Insert 4 Well is not working on wet or moist surfaces. It might also not work on uneven or dusty substrates.

**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  $\mu$ 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 700  $\mu$ 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  $\mu$ -Dish<sup>35mm, 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 4 Well, use a smaller seeding density to create a less confluent cell layer or decrease incubation time.

**Immersion Oil**

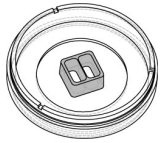
When using oil immersion objectives, use only the immersion oils specified in the table. The use of a non-recommended oil could lead to the damage of the plastic material and the objective.

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

**Ordering Information**

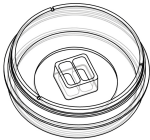
The Culture-Insert is available with different numbers of wells and in various product versions.

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

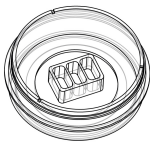


Cat. No.	Description
80206	<b>Culture-Insert 2 Well in <math>\mu</math>-Dish<sup>35mm, low</sup>, ibiTreat:</b> ready to use, tissue culture treated, sterilized

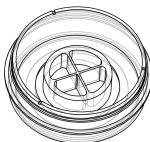
Culture-Insert in  $\mu$ -Dish<sup>35mm, high</sup>



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

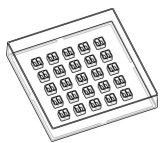


80366	<b>Culture-Insert 3 Well in <math>\mu</math>-Dish<sup>35mm, high</sup>, ibiTreat:</b> ready to use, tissue culture treated, sterilized
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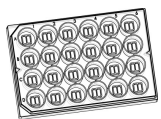
80466	<b>Culture-Insert 4 Well in <math>\mu</math>-Dish<sup>35mm, high</sup>, ibiTreat:</b> ready to use, tissue culture treated, sterilized
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25 Culture-Inserts for self-insertion



Cat. No.	Description
80209	<b>25 Culture-Inserts 2 Well for self insertion:</b> in a 10 cm transport dish, sterilized
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 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](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. All products are developed and produced in Germany.

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