# Instructions





The Culture–Insert is a product for well-defined cell seeding. A special sticky and biocompatible surface at the bottom side works like a glue and avoids leaking. A cell suspension can be placed in one or both 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. Only the attached cells grow on one or two spots. The Culture-Inserts can be placed on every flat, clean, and dry surface. After cell attach-

ment it can be removed without remains. When both wells are filled with adherent cells, a cell-free gap of approx. 500 µm is created after removing the Culture-Insert. It is intended for wound healing assays, co-cultivation, invasion or chemotaxis assays. Several other applications are possible.

#### Material

The product is manufactured from biocompatible silicone material. Although, the material is autoclavable and compatible to alcohols we do not recommend reusing it.

## **Geometry of the Culture-Inserts**

Geometry of the Culture-Insert			
Number of wells	2		
Outer dimensions (w $\times$ l $\times$ h)	$9 \text{ mm} \times 9 \text{ mm} \times 5 \text{ mm}$		
Growth area per well	$0.22 \text{ cm}^2$		
Recommended filling volume per well	70 µl		
Width of cell-free gap	$500 \ \mu m \pm 50 \ \mu m$		

We recommend using the Culture-Inserts in ibidi  $\mu$ -Dishes, ibidi  $\mu$ -Slide 8 well, 6 well plates, 12 well plates or petri dishes. It is also possible to use them on sterile glass coverslips or glass slides.

## Surfaces and coatings

We recommend using the Culture–Inserts 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–Inserts can be transferred to any flat, clean, and dry surface. Use sterile tweezers for transfer and gently push. Keep in mind that only the bottom side is sticky.

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

## Seeding cells

- Prepare cell suspension as usual. 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 medium. Use the recommended volume of the dish minus 200 µl.
- After appropriate cell attachment (24 hours) gently remove the Culture–Insert by using sterile tweezers.
- Fill the used well or dish with cell free medium. Use the recommended volume (e.g. for µ-Dish <sup>35mm, high</sup> use 2 ml).
- Conduct your experiment.





#### Tip:

Wound healing assays using ibidi Culture–Inserts 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.

In case the cell lawn is (partially) removed together with the Culture–Insert use a smaller seeding density to create a less confluent cell layer or decrease incubation time.

#### Culture–Insert family

The Culture–Insert is available in different product versions.

#### Culture Insert, µ–Dish <sup>35mm, low</sup>

	Ordering number	Treatment	Characteristics
	80206	ibiTreat, sterile	hydrophilic, in μ–Dish <sup>35mm, low</sup>
	80201	uncoated, sterile	hydrophobic, in μ–Dish <sup>35mm, low</sup>

#### Culture Insert, µ–Dish 35mm, high

	Ordering number	Treatment	Characteristics
	81176	ibiTreat, sterile	hydrophilic, in μ–Dish <sup>35mm, high</sup>
	81171	uncoated, sterile	hydrophobic, in µ–Dish <sup>35mm, high</sup>

#### 25 Culture-Inserts for self insertion

	Ordering number	Treatment	Characteristics
	80209	no direct use, sterile	for self insertion, in transport dish

#### Culture–Insert 24

	Ordering number	Treatment	Characteristics
	80241	tissue culture treated polystyrene*, sterile	hydrophilic, in 24 well plate
	*This plate is made of PS which is not suitable for fluorescence or high resolution microscopy.		

#### 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. © ibidi GmbH, Am Klopferspitz 19, 82152 Martinsried, Germany.