The ibidi Culture–Insert 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 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. When both wells are filled with adherent cells, a cell–free gap of approx. 500 µm is created after removing the Culture–Insert. The Culture–Insert is also intended for 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 × l × h)	9 mm × 9 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

We recommend using the Culture–Inserts in ibidi µ–Dishes, µ–Slide 2 well, µ–Slide 4 well or µ–Plate 24 well. The Culture–Inserts 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.

Surfaces and Coatings

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

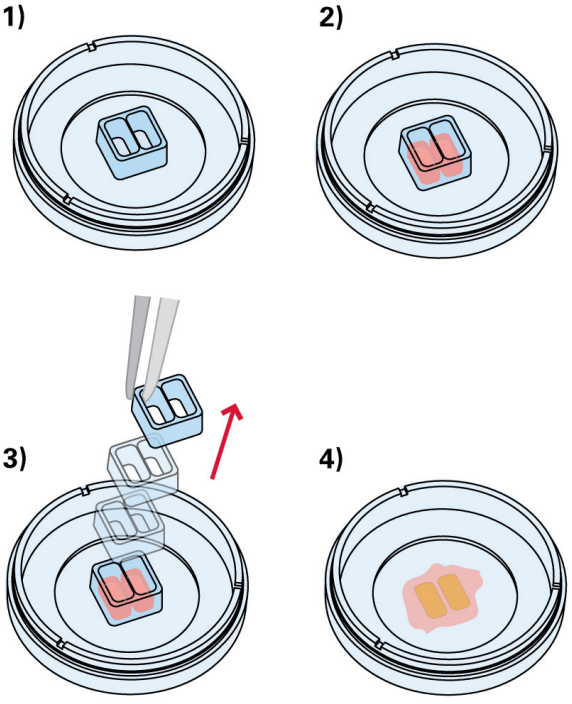
Please test the compatibility with your specific protein coating with a free sample available on www.ibidi.com.

The Culture–Inserts can be transferred to any flat, clean, and dry surface. Use sterile tweezers for transfer and gently push 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 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. Grab a corner of the Culture Insert.
- Fill the used well or dish with cell free medium. Use the recommended volume (e.g. for µ–Dish $^{35mm, high}$ use 2 ml).
- Conduct your experiment.



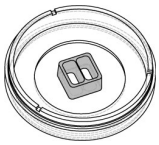
Tip:

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

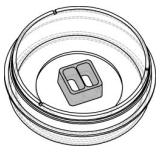
The Culture–Insert is available in different product versions.

Culture Insert, µ–Dish $^{35mm, low}$



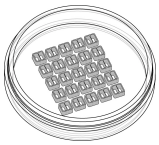
Ordering number	Treatment	Characteristics
80206	ibiTreat, sterile	hydrophilic, in µ–Dish $^{35mm, low}$
80201	uncoated, sterile	hydrophobic, in µ–Dish $^{35mm, low}$

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



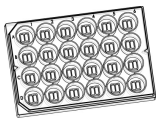
Ordering number	Treatment	Characteristics
81176	ibiTreat, sterile	hydrophilic, in µ–Dish $^{35mm, high}$
81171	uncoated, sterile	hydrophobic, in µ–Dish $^{35mm, high}$

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.

Selected References

J. Behrens, P. Kameritsch, S. Wallner, U. Pohl, and K. Pogoda. The carboxyl tail of Cx43 augments p38 mediated cell migration in a gap junction-independent manner. *European Journal of Cell Biology*, 2010. doi: 10.1016/j.ejcb.2010.06.003.

R. Djafarzadeh, M. Sauter, S. Notohamiprodjo, E. Noessner, P. Goyal, W. Siess, M. Wörnle, A. Ribeiro, S. Himmelein, T. Sitter, and P. J. Nelson. Recombinant GPI-Anchored TIMP-1 Stimulates Growth and Migration of Peritoneal Mesothelial Cells. *PLoS ONE*, 2012.

A. Msaki, A. M. Sanchez, L. F. Koh, B. Barre, S. Rocha, N. D. Perkins, and R. F. Johnson. The Role of RelA (p65) Threonine 505 Phosphorylation in the Regulation of Cell Growth, Survival, and Migration. *Molecular Biology of the Cell*, 2011. doi: 10.1091/mbc.E11-04-0280.

Y.-T. Shih, M.-C. Wang, H.-H. Peng, T.-F. Chen, J.-Y. Chang, and J.-J. Chiu. Modulation of Chemotactic and Pro-Inflammatory Activities of Endothelial Progenitor Cells by Hepatocellular Carcinoma. *Cellular Signalling*, 2012. doi: 10.1016/j.cellsig.2011.11.013.

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