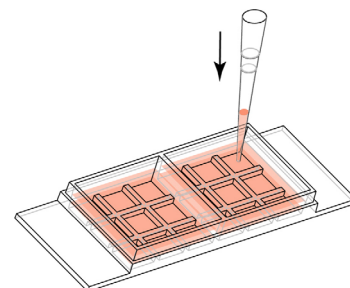
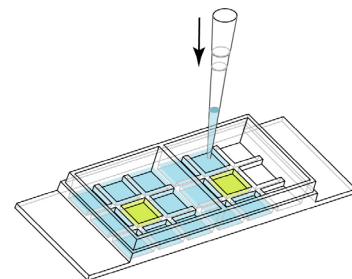
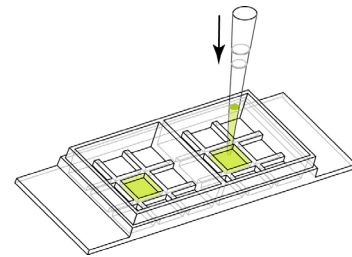


## Co-Cultivation Using ibidi $\mu$ -Slides

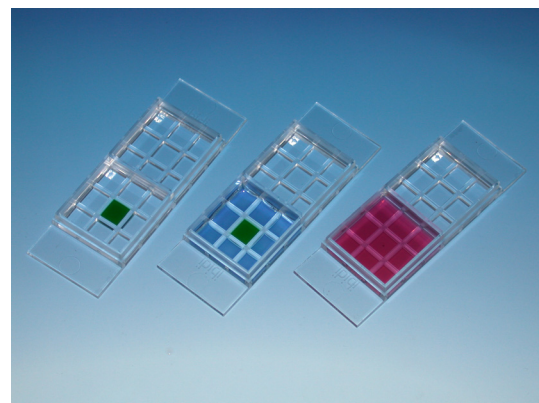
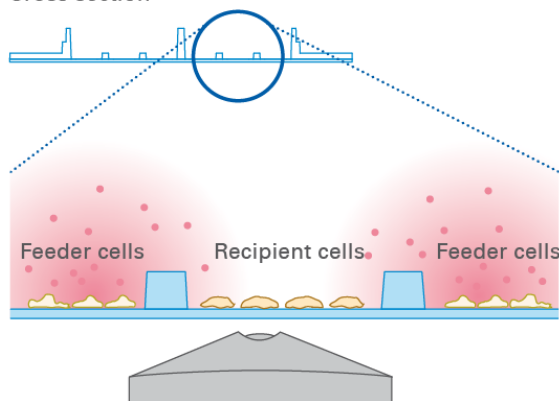
In this example we show how the  $\mu$ -Slide 2 x 9 well can be used for co-cultivation of two different cell types. Feeder and recipient cells can be grown individually but sharing the same medium to communicate by soluble factors/proteins.

### General Protocol for the $\mu$ -Slide 2 x 9 well

1. Unpack the  $\mu$ -Slide 2 x 9 well and place it on a  $\mu$ -Slide Rack or another appropriate surface. Prepare your recipient cells and seed them into the center minor well using 40-60  $\mu$ l cell suspension. Depending on your cells we recommend  $5-10 \times 10^4$  cells/ml.
2. Prepare your feeder cells and seed them into the outer minor wells using 40-60  $\mu$ l cell suspension for each well. When using the ibiTreat (hydrophilic – tissue culture treated) surface some mixing between the outer 8 wells may occur. Don't wet the catwalks of the inner well with medium and handle the slide with care not to mix the media before the cells have attached.
3. After cell attachment, empty the individual reservoirs to prevent cell mixing. Wash the 9 minor wells with 40-60  $\mu$ l medium to remove non adherent cells (not shown). After that, fill 400-600  $\mu$ l medium into each large well. This will connect the 9 minor wells allowing the two cell types to communicate via the supernatant.



Cross section

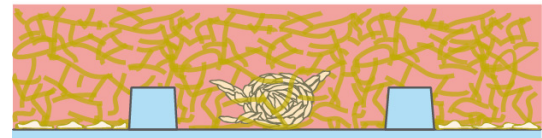
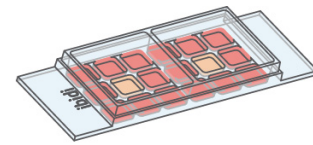


## Application Note 10

### Modifications and Different Assays for Co-Cultivation of Cells

#### μ-Slide 2 x 9 well with Multicellular Spheroids and/or 3D Gel Matrices

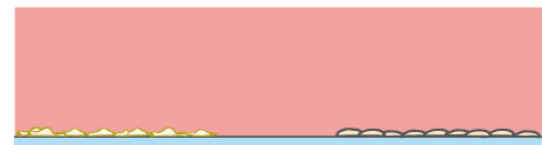
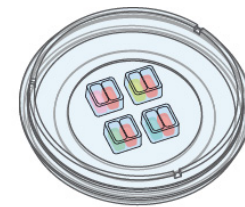
The **μ-Slide 2 x 9 well** also allows for the cultivation of cell spheroids inside a gel matrix, in combination with feeder cells seeded in the outer wells. In the picture on the right, a multicellular spheroid of e.g. endothelial cells is embedded into a collagen gel. Growing in the center minor well the spheroid can be cultured next to cancer cells which can release soluble factors. Aqueous 3D gel matrices like Collagen I gels do not hinder molecule diffusion.



Cell Type 1 Cell Type 2

#### Co-Cultivation in 2D with ibidi Culture-Insert

The ibidi **Culture-Insert** can be used for plating different cell types into one culture vessel, next to each other. It is used as a stencil for seeding cells only in the designated areas. After removing the Culture-Insert the different cell types grow directly next to each other without a barrier. In contrast to the μ-Slide 2 x 9 well, individual cell patterns can be created. Depending on the plate or Petri dish used, the number of cells and volume of culture medium can be chosen individually.



Cell Type 1

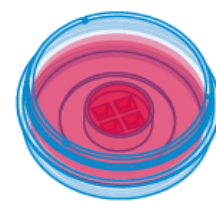
Cell Type 2

#### Co-Cultivation of Cells in a 3D Matrix

The ibidi **micro-Insert 4 well** provides small wells which can be filled with cells that are mixed into a 3D gel matrix. This way, different types of suspension or adherent cells can be cultured individually while sharing one supernatant. This supernatant can be collected or exchanged as desired without flushing the cells out of the gel.



Gel + Cells



Filling the μ-Dish

