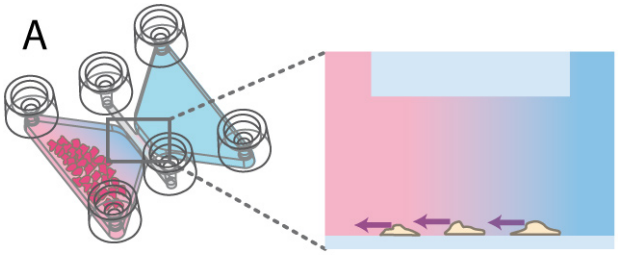
Chemotaxis Assay with Cells Producing a Chemoattractant

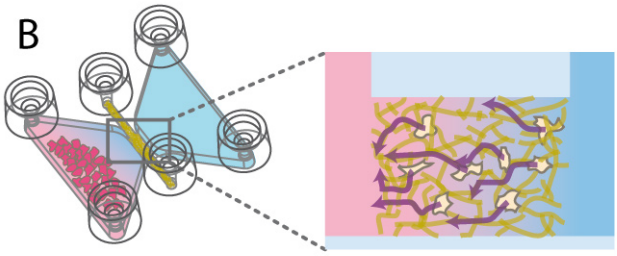
General information: In this Application Note, we present a special protocol how to use chemoattractant producing cells to promote chemotaxis of another cell type. This Application Note is a specialized protocol of Application Note 17 in which the standard use of the µ-Slide Chemotaxis is described. AN 17 contains more detailed information about slide handling, experimental planning and data analysis.

Table of Contents

1. Background Information

Chemotaxis assays often utilize supernatants, conditioned medium or secreted factors as chemoattractants. With the chemotaxis assay using µ-Slide Chemotaxis 3D it is possible to use any solution as chemoattractant. By following the protocol below it is possible to seed cells in the large reservoirs. By segregating factors the seeded cells can act as a chemoattractant for the cells in the center. This protocol is identical for 2D and 3D assays.





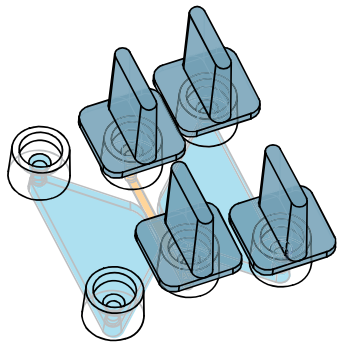
Important Notes: Detailed µ-Slide Handling, experimental planning and data analysis is provided in Application Note 17.

Principle of cells producing a chemoattractant in one of the large reservoirs of µ-Slide Chemotaxis. The cells which are tested for a chemotactic movement must be placed in the observation area in the center of the µ-Slide Chemotaxis. The cells producing a chemoattractant are seeded into one of the large reservoirs. The released factors will create a stable gradient over the observation area.
A) Chemotaxis assay in 2D.
B) Chemotaxis assay in 3D with a gel matrix for the migrating cells.

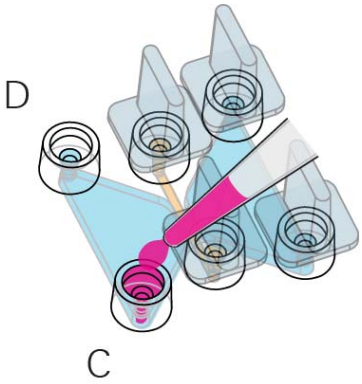
2. Equipment Needed

The necessary equipment for this assay is described in detail in Application Note 17. For this special protocol only one additional cell type is necessary.
As described in Application Note 17, please keep in mind that you will need compatible pipet tips, slant tweezers, a humid chamber and a heating and incubation system for optimal conditions for your cells.

3. Protocol

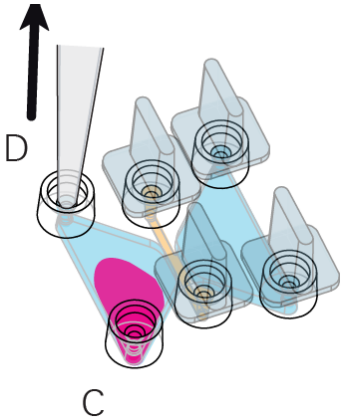


- Follow the protocol of Application Note 17 just until the step before the chemoattractant is added. It does not matter whether the migrating cells are seeded as a monolayer (2D) or embedded into a gel matrix (3D).



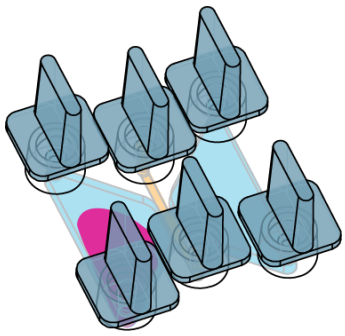
- Prepare the cell suspension of the chemoattractant-producing cells. Adjust the cell concentration to 1-5 x 10^5 cells/ml.

- Use a 20 µl pipet (e.g. Gilson P-20) and apply 15 µl cell suspension to the top of Filling Port C, as shown. Do not inject directly.



- Use the same pipet settings and aspirate 15 µl air from the opposite Filling Port D. Press the pipet tip directly into Filling Port D. The cell suspension on top of Filling Port C will be flushed inside and fill the reservoir.

- Repeat this step one once, in order to fill 30 µl of the cell suspension inside the reservoir.



- Gently close all Filling Ports with plugs to hinder evaporation. Handle with care.

- Let the cells sediment and attach for 30 minutes.

- Start video microscopy

4. Notes

4.1 Control Experiments

Keep in mind to conduct two control experiments in which you:

1) Seed no cells as chemoattractant. (Control experiment -/-)
2) Seed cells as chemoattractant in both reservoirs. (Control experiment +/+)

4.2 Position of Chemoattractant-producing Cells and Migrating Cells

The migrating cells must be placed in the observation area in the center of the µ-Slide Chemotaxis. A stable gradient can only be created there.
The cells which shall produce a chemoattractant must be seeded inside the large reservoirs.

4.3 Growth Media of the two Cell Types

Using the same growth medium for both cell types is highly recommended for this experiment. Different media might hinder the establishment of a gradient due to different densities causing convection inside the chamber.

4.4 Surfaces and Coatings

For adherent chemoattractant producing cells the surface might be crucial for attachment and production of chemoattractants. Both product versions of the µ-Slide Chemotaxis, 80326 (ibiTreat) and 80322 (Collagen IV) provide the ibiTreat surface inside the large reservoirs. Only the observation area is coated in the Collagen IV version.
To apply a coating on the ibiTreat surface in the large reservoirs, please follow the coating protocol in Application Note 17.
If no special coating is necessary, the tissue culture treated ibiTreat surface provides excellent cell attachment for adherent cells.

4.5 Serum Concentration

For optimal attachment of the chemoattractant producing cells, the optimal serum concentration is recommended. For the chemotaxis experiment itself, a lower serum concentration is recommended to reduce growth factors which might mask the chemotaxis effect.

4.6 Optimization

For better results the following parameters can be changed:

1) Cell seeding concentration of the chemoattractant-producing cells
2) Surface treatment/coating of the chemoattractant-producing cells
3) Matrix/Surface of the migrating cells
4) Experiment duration
5) Serum concentration