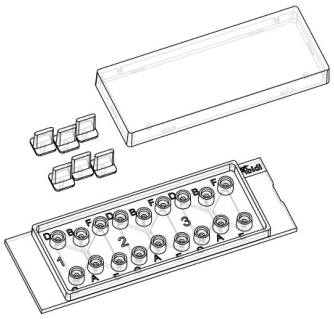


The ibidi product family comprises a variety of different shapes of µ–Slides and µ–Dishes which all have been designed for high–end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

The µ–Slide Chemotaxis 3D is a tool for investigation of chemotaxis and migration of non-adherent cells in gel matrices. The chamber's geometry is optimized for analyzing chemotaxis by video microscopy. The linear concentration profile which is required for chemotactical movement is generated by diffusion through aqueous gels and stable for at least 48 hours.

Please read the following Application Notes for more detailed information:

Application Note 17 "3D Chemotaxis Assays using µ–Slide Chemotaxis 3D": This AN contains a general protocol for 3D gel assays with µ–Slide Chemotaxis 3D. There is also detailed handling information.

Application Note 23: "3D Chemotaxis Protocol with Collagen I Gel for Dendritic Cells": This AN provides an example protocol for chemotaxis of Dendritic cells in a collagen gel.

Application Note 26: "Fabrication of Collagen I Gels": This AN contains protocols for collagen Type I gels with different concentrations and different culture media.

Material

ibidi µ–Slides, µ–Dishes, and µ–Plates consist of a plastic with highest optical quality. The bottom material exhibits extremely low birefringence and autofluorescence, both similar to that of glass. It is not possible to detach the bottom from the upper part. The µ–Slides, µ–Dishes, and µ–Plates are not autoclavable since they are temperature stable up to 80°C/175°F only. Please note that gas exchange between the medium and incubator's atmosphere occurs partially through the plastic bottom which should not be covered.

Optical Properties ibidi Standard Bottom	
Refractive index n_D (589 nm)	1.52
Abbe number	56
Thickness	No. 1.5 (180 µm)
Material	microscopy plastic

Coating your µ–Slide Chemotaxis 3D

Detailed information about coatings is provided in Application Note 08 "Cell culture coating".

[1] Collagen IV, BD Cat.-Nr. 35 6233

Geometry

Geometry µ–Slide Chemotaxis 3D	
Chambers on slide	3
Volume per chamber	130 µl
Observation area	2 × 1 mm^2
Distance between chambers	18.5 mm
Total height with plugs	12 mm
Volume chemoattractant	30 µl
Bottom matches coverslip	No. 1.5

µ–Slide surfaces

Depending on your cells and special application you will need µ–Slides with different surfaces.

ibiTreat is a tissue culture treated surface. The ibiTreat surface is very hydrophilic which facilitates filling the structure with aqueous gels for 3D assays.

The Collagen IV precoated slides are surface coated only. The Collagen IV precoated slides do not contain a collagen gel. The surface of the observation area is coated with Collagen IV to mediate cell adhesion for possible 2D experiments. Only high quality substrates are used [1].

Seeding cells

Detailed information on correct slide handling is provided in Application Note 17 "3D Chemotaxis Assays using µ–Slide Chemotaxis 3D".

Here are the short steps for cell seeding and conducting chemotaxis experiments:

1. Prepare your cell suspension as usual. Use cell suspension of approx. 3 $\times 10^6$ cells/ml (final in gel).
2. Bring cell suspension into a gel*.
3. Close filling ports of the large reservoirs by plugs.
4. Apply 6 µl gel onto one filling port of the side channel. Do not inject the gel directly.
5. Aspirate 6 µl of air from the opposite filling port. The gel–cell mixture will be flushed into the channel.
6. Close the two filling ports of the channel.
7. Remove all plugs from the filling ports of the large reservoirs.
8. Incubate the slide inside a sterile and humid atmosphere to minimize evaporation until the gel is formed. Make sure evaporation is low by using a sterile 10 cm Petri dish with extra wet tissue around the slide.

Important!

The day before seeding place the cell medium and the µ–Slide into the incubator for equilibration. This will prevent the liquid inside the channel from emerging air bubbles over the incubation time.

Chemotaxis experiment

1. After the gel matrix is solidified fill both reservoirs with 65 µl chemoattractant–free medium.
2. Fill 2 ×15 µl chemoattractant solution into one reservoir.
3. Close all filling ports with plugs.
4. Conduct video microscopy.
5. Track cells and analyze migration. Please visit www.ibidi.com for a software tool analyzing migrational data.

Cell seeding and conducting a chemotaxis experiment is described in detail in Application Note 17.

Perform control experiments (+;+) and (-;-) similarly.

Preparation for Cell Microscopy

To analyze your cells no special preparations are necessary. Cells can be observed live or fixed directly in the µ–Slide on an inverted microscope. You can use any fixative of your choice. The µ–Slide material is compatible with a variety of chemicals, e.g. Acetone or Methanol. Further specifications can be found at www.ibidi.com. Due to the thin bottom of only 180 µm, high resolution microscopy is possible.

Troubleshooting

Tips and tricks on handling and further troubleshooting is provided in Application Note 17 "3D Chemotaxis Assays Using µ–Slide Chemotaxis 3D".

Immersion Oil

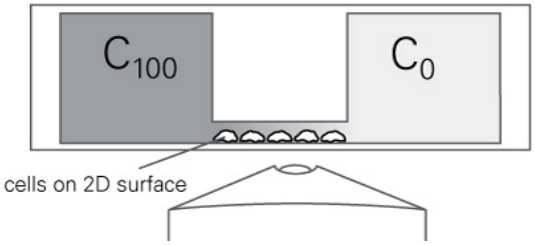
When using oil immersion objectives, only the immersion oils specified in the table may be used. The use of different oil can lead to damages of the plastic material and the objective.

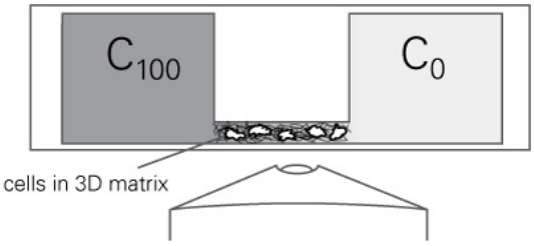
Company	Product	Ordering number
Zeiss	Immersol 518 F	(Zeiss) 444960
Zeiss	Immersol W 2010	(Zeiss) 444969
Leica	Immersion liquid	(Leica) 11513859

*Please find example protocols here:
Application Note 23 "3D Chemotaxis Protocol with Collagen I Gel for Dendritic Cells"
Application Note 26 "Fabrication of Collagen I Gels"

µ-Slide Chemotaxis selection guide

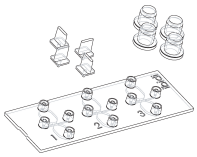
µ–Slide Chemotaxis 2D	µ–Slide Chemotaxis 3D
Migration of adherent cells on a flat surface.	Migration of non–adherent cells in 3D gel matrix. Can also be used for a 2D chemotaxis experiment with adherent cells.
For slow migrating, adherent cells, e.g. endothelial cells, cancer cells or fibroblasts.	For fast or slow migrating cells embedded in a 3D gel matrix (or adherent cells, respectively) e.g. neutrophils, lymphocytes and dendritic cells. A gel matrix is not part of the product.
Gradient is long term stable.	Gradient is long term stable.





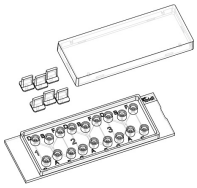
µ–Slide Chemotaxis family

µ–Slide Chemotaxis 2D



Ordering number	Treatment or Coating	characteristics
80306	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80302	Collagen IV, sterile	protein coating

µ–Slide Chemotaxis 3D



Ordering number	Treatment or Coating	characteristics
80326	ibiTreat, tissue culture treated, sterile	hydrophilic, tissue culture treated
80322	Collagen IV, sterile*	protein coating

* Surface coating of observation area. Does not contain a gel matrix.

Selected References

Y. Harada, Y. Tanaka, M. Terasawa, M. Pieczyk, K. Habiro, T. Katakai, K. Hanawa-Suetsugu, M. Kukimoto-Niino, T. Nishizaki, M. Shirouzu, X. Duan, T. Uruno, A. Nishikimi, F. Sanematsu, S. Yokoyama, J. V. Stein, T. Kinashi, and Y. Fukui. Dock8 is a cdc42 activator critical for interstitial dendritic cell migration during immune responses. *Blood*, 2012. doi: 10.1182/blood-2012-01-407098.

C. Ocana-Morgner, P. Reichardt, M. Chopin, S. Braungart, C. Wahren, M. Gunzer, and R. Jessberger. Sphingosine 1-phosphate-induced motility and endocytosis of dendritic cells is regulated by swap-70 through rhoa. *The Journal of Immunology*, 2011. doi: 10.4049/jimmunol.1003461.

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 46 17 0. All products are developed and produced in Germany.