MICROCHANNELS DISH

Material included

Microchannels dishes

In option:

Extracellular matrix protein

Extra material recommended not included in the kit

Micropipette 2-20 µL
PBS solution

The microchannels dishes are sent in individual sterile plastic bags. The chips in PDMS are bonded to glass bottom petri dishes, the channels against the glass bottom. Make sure to manipulate in sterile conditions.

A. WASHING THE MICROCHANNELS

1. In sterile conditions, open the individual sterile plastic bag which contains the dish.
2. Wash the dishes by immerging the PDMS in 3 mL of PBS. Remove it either with an aspirating pump or by flipping the dish. Ensure not to aspirate directly inside the chambers to prevent from drying.
3. Repeat this step twice but let the PBS incubate for 5 minutes before removing it.

B. ADHESIVE SOLUTION COATING

1. Discard 10 µL of solution from each chamber.
2. Fill the entry of the channels with 10 µL of surface coating solution[(1)] per access port.
3. Incubate 1 hour at room temperature to allow adsorption of the coating substrate[(2)].

C. PLATING THE CELLS

1. After the fibronectin coating, extensively wash the device with PBS 3 times as explained previously.
2. Similarly, rinse 3 times the channels by immerging them into 2 mL of culture medium pre-heated at 37°C [(3)(4)]. Incubate in the medium at 37°C for 15 minutes[(5)].
3. Discard the medium either with an aspirating pump or by flipping the dish, and discard 10 µL of solution from each access port.
4. Place a droplet of cell solution (5 µL or 10 µL) per access port. Cell concentration should be adjusted to reach a confluency of 60-70% inside the hole. To achieve this, we advise a cell concentration ranging from 10^6 to 10^7 cells/mL.[(6)]
5. Close the lid of the Fluorodish. Place the Fluorodish into a humidified incubator with the appropriate settings for the cell type used (typically for mammalian cells this will be 37°C and 5% CO_2) to allow cell adherence. Incubate at 37°C for 30 minutes to one hour.
6. Add 2 mL of culture medium[(3)].
7. Incubate at 37°C, 5% CO_2 at least 4 hours[(7)] before observing the cells into the channels.

(1) 4Dcell fibronectin solution is provided at a concentration of 50 µg/mL. Fibronectin is standardly used at 10 µg/mL, but the concentration may be adapted depending on the cell type used and the results expected. Other substrates such as collagen, PEG, ... may be used to modify cell adherence to the channel walls.

(2) The solution should infiltrate the channels by capillary forces: make sure that the liquid spreads throughout the entire structure. This can be easily checked by eye under regular light or using a regular bright field microscope. In very small structures, in which diffusion is harder, entry of liquid in the channels can be forced by placing the structure in a vacuum jar bell during at least 15 min. Alternatively, the protein solution can be incubated overnight at 4°C.

(3) The whole PDMS structure must be covered. If 2 mL is not enough, add more medium to completely cover it.

(4) For experiments involving drugs like molecule inhibitors, it is advised to preincubate the channels with a medium containing the drug at the right concentration.

(5) The PDMS absorbs molecules in the medium. So if you have fragile cells, an overnight incubation with PBS (3 mL) at 37°C is recommended before seeding the cells.

(6) High cell density is required to stimulate the contact of the cells with the channels. Low cell density may result in low number of cells inside channels and failure of the experiment. If needed, apply an "up & down" movement with the pipette to push the cells towards the borders.

(7) This duration will depend on the cell characteristics and migratory capacities.

Reference: Vargas P., Terriac E., Lennon-Duménil AM., Piel M., "Study of cell migration in microfabricated channels", J Vis Exp. 2014 Feb 21;(84):e51099. DOI: 10.3791/51099.
Vargas P., Chabaud M., Thiam HR., Lankar D., Piel M., Lennon-Dumenil AM., "Study of dendritic cell migration using micro-fabrication", J Immunol Methods. 2016 May;432:30-4. doi: 10.1016/j.jim.2015.12.005. Epub 2015 Dec 9.
Vargas P., Saez P., Barbier L., Attia R., Thiam H., Piel M., "Leukocyte migration and deformation in collagen gels and microfabricated constrictions", in Cell Migration: methods and protocols, Methods in Molecular Biology 1749 edited by Alexis Gautreau, Laboratory of Biochemistry, Ecole Polytechnique, Palaiseau, France, 2018, pp. 368-369

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