MICROPATTERNED SLIDES

Material included

Micropatterned slides

In option:

Extracellular matrix protein

Recommended material

Micropipette 20-200 µL
PBS solution
Distilled water
Kimwipes
Parafilm
Plastic tweezers

The micropatterned slides are sent inside standard 35 mm petri dishes, patterns facing upwards.
Make sure to manipulate in sterile conditions.

A. ADHESION PROTEIN INCUBATION

1. In sterile conditions, open the individual sterile plastic bag which contains the petri dish.
2. On a parafilm, pipette 100 µL of the fibronectin solution [1][2].
3. Carefully take the slide with plastic tweezers and place the micropatterned side of the slide on the drop of fibronectin.
4. Incubate 1 hour at room temperature.
5. Rinse the slide by gently placing it on a big drop of water. To dry the slide, drop off the last drop on a Kimwipes and place it on a slide rack. Let it air dry.

B. PLATING THE CELLS

1. Rinse cells once with PBS.
2. Detach cells, if possible with EDTA 0.02%, if not, with diluted trypsin. Centrifuge to get rid of the supernatant.
3. Resuspend cells in 1 mL of the culture medium for each slide: mix extensively with a 1 mL pipette to separate the cells from each other. Then you can add additional medium.
4. Deposit 100 000 to 200 000 cells for each 24 mm slide [3]: place your slide in a petri dish, with the patterned side oriented face to you. Put the cells in their culture medium onto the slide with the micropipette.
5. Wash twice unattached cells 30 minutes to 1 hour (depending on the cell type) after plating with equilibrated hot medium. [4]
   Cells should be spread on the patterns 2 to 4 hours after plating. Usually about 70% of cells are attached and spread on the micropatterns. Make sure to place your cells in the incubator at 37°C with 5% CO_2 in between the different steps.

(1) The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type.
(2) It can also be mixed with fluorescent fibrinogen at a concentration of 20 µg/mL at a ration of 30 µL of fibrinogen with 70 µL of fibronectin. This will allow you to see the patterns in fluorescence.
(3) It is better to have a confluency around 50%. High confluency of cells will promote cell-cell adhesion and give you clusters of cells, which is bad for single cell patterning.
(4) An efficient washing is obtained by adding medium from one side and aspirate from the other side at the same time.

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