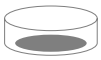
GELS

Glass-bottom Petri dishes coated with polyacrylamide gels

Materials included:



Glass-bottom Petri dish coated with polyacrylamide gel functionalized with fibronectin

Materials not included:

100-1000 µL micropipette
PBS

Before starting:

- The gels are provided already coated with fibronectin (for cell adherence)
- Always employ aseptic techniques to maintain sterility of coverslips and other materials
- The gels are shipped provided in a water-based solution. Keep unused gels at 4°C (expiration date is one month from the date of delivery)
- Keep the gels wet at all time
- Please read the notes displayed on the second page carefully before initiating the experiment

A. UNPACKAGING

1. The gel coated Petri dishes should be placed at room temperature for 30 minutes before use
2. Open the individual plastic bag containing the Petri dish inside a laminar flow hood. The lid is placed under the Petri dish.
3. Remove the adhesive layer which is covering the gel coated Petri dish.
4. With the help of a pipette, safely discard the water-based solution.

B. SEEDING CELLS

1. Rinse the gel coated Petri dish twice with sterile PBS.
2. Add 2 mL of pre-warmed cell culture medium to the gel coated Petri dish and incubate for 15 minutes at 37°C, 5% CO_2, 95% humidity (gel equilibration).
3. Meanwhile, resuspend 200,000 to 300,000 cells in 2 mL of cell culture medium per Petri dish[(1)].
4. At the end of the gel equilibration period (15 minutes), remove as much medium as possible from the Petri dish.
5. Pipet drop by drop 2 mL of cell suspension on top of the gel (throughout the whole gel surface) and incubate for at least 1 hour at 37°C, 5% CO_2, 95% humidity. Check if cells are attached before proceeding to step 6[(2)].
6. Remove medium with unattached cells. Do not touch the gel with the pipette tip[(3)].
7. Wash gel once with 3 mL of pre-warmed medium (add the medium on the edge of the Petri dish, not on top of the gel).
8. Discard the medium.
9. Add 2 mL of pre-warmed culture medium per Petri dish and incubate cells overnight at 37°C, 5% CO_2, 95% humidity for future analysis[(4)].

4Dcell
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NOTES

(1) Please note that the number of cells depends on the confluence you want to attain, you should optimize the process according to your experiments and cell type used. See following note (number 2) before pipetting the cell solution on the gel.

(2) Do not pipet the cell solution only in the center of the gel to avoid formation of clusters and poor spread of the cells. Since the gel is wet, some of the cells will attach between the gel and the edges of the Petri dish. Please take that into account when optimizing the number of cells you need per gel. The gel surface diameter is 18 mm, and the Petri dish has a diameter of 35 mm.

Some cell types take longer than 1h to attach to the gel. Check cell attachment to the gel on the microscope by gently shaking the Petri dish. At this stage, if the cells are attached they will not move on top of the gel.

(3) Tilt the Petri dish and aspirate the medium containing unattached cells by placing the pipette tip on the edge of the Petri dish.

(4) The cell seeding is optimized for HeLa cell line. Therefore, the number of cells added, the adhesion, and/or culture times can vary. Make sure the gels do not dry at any moment during the protocol.