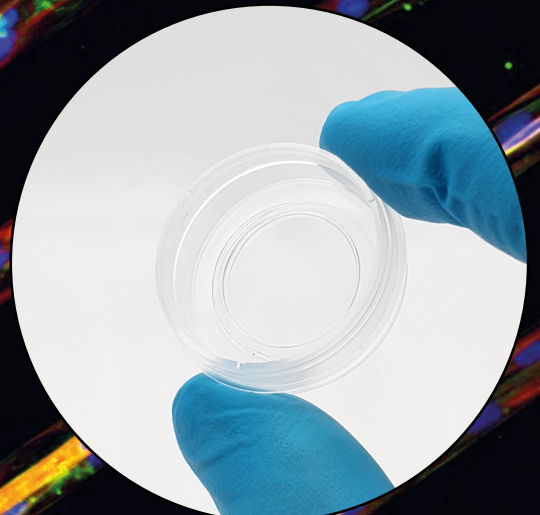


**4Dcell micropatterned Petri dishes**  
User protocol

**USER GUIDE**



# 4Dcell micropatterned Petri dishes User Guide

## How to use 4Dcell micropatterned Petri dishes

### Materials included

Micropatterned glass Petri dishes

### Materials not included

100-1000  $\mu$ L micropipette  
Distilled water  
PBS

### Optional

> **Coating solution** (e.g. fibronectin, collagen, laminin).

*The fibronectin solution provided by 4Dcell has a concentration of 50  $\mu$ g/mL. It can be adjusted according to the cell type.*

> **Fluorescent-labelled protein** (e.g. fibrinogen, BSA, collagen, laminin).

*The patterns on the Petri dish can be visualized using fluorescent fibrinogen or other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20  $\mu$ g/mL.*

### Shipping & storage

Petri dishes coated with oxidation resistant polymer can be stored up to 6 months at room temperature from the date of delivery.

### Before starting

To avoid contaminations, only manipulate the coverslips using aseptic techniques in a laminar flow hood.

The micropatterns consist of a thin layer of polymer bound to the surface of the glass. To avoid scratching this layer, pipette solutions into the Petri dish carefully and avoid touching the surface of the glass.

The timing of protein and/or cell incubation can vary depending on your needs. The protocol suggested by 4Dcell was optimized for HeLa cells. You may need to adapt this protocol for your cell type.

Incubation with an adhesion protein is optional. We suggest only using an adhesive protein if your cell type does not readily adhere to glass substrates. Several adhesive coating proteins can be employed if needed (fibronectin, laminin or collagen). You can also try pre-incubating the dishes in cell medium (media typically contain proteins which can help adhesion).

## Protocol

**a.** Open the individual plastic bag containing the Petri dish using aseptic technique in a laminar flow hood.

**If coating the Petri dishes with an adhesion protein is necessary, proceed to step a.1. Otherwise, proceed directly to step b.**

**a.1.** *Optional: Pipette 400  $\mu$ L of your adhesive protein in each Petri dish<sup>(1)/(2)</sup>.*

**a.2.** *Optional: Incubate for 30 min to 1 hour at room temperature.*

**a.3.** *Optional: Discard the adhesive protein solution from the Petri dish.*

**a.4.** *Wash the Petri dish 3x with 1 mL of distilled water.*

**b.** Prepare a cell solution. For a standard design, we recommend 30 000 – 60 000 cells resuspended in up to 400  $\mu$ L of cell culture medium<sup>(3)</sup>. For some patterns (for instance wound healing), we recommend a higher density of cells.

**c.** Pipet 400  $\mu$ L of cell suspension into each coverslip.

**d.** Carefully transfer the Petri dishes from the hood to the incubator<sup>(4)</sup>.

**e.** Incubate the micropatterned Petri dish containing the cells at 37°C, 5% CO<sub>2</sub>.

**f.** *Optional:* after the initial adhesion (30 minutes - 2 hours, depending on the cell type), carefully remove the medium to remove unadhered or poorly adhered cells. Tilt the petri dish and allow the medium to flow into the edge of the petri dish. Remove the medium and replace with fresh warm medium in the corner of the petri dish to avoid perturbing the newly adhered cells. The cells can also be rinsed with PBS solution before adding warm medium<sup>(5)</sup>.

**g.** Patterned cells can be observed under the microscope after several hours of incubation.

## Notes

(1) The fibronectin solution provided by 4Dcell has a concentration of 50  $\mu$ g/mL. It can be adjusted according to the cell type. The incubation with fibronectin can be performed directly on the coverslips attached to the Petri dish (higher volume of protein solution is need to cover the whole glass coverslip).

(2) The patterns can be visualized using fluorescent fibrinogen or other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20  $\mu$ g/mL.

(3) The seeding density is optimized for HeLa cells. The number of cells added and the adhesion or culture times could vary for other cell types. Make sure the cell suspension is thoroughly re-suspended into individual cells before pipetting into the wells. It is better to re-suspend cells that are at a confluency around 50% or less. High confluency of cells before re-suspension and seeding onto the plate will promote cell-cell adhesion and cell cluster formation, which is not suitable for single cell patterning.

(4) Avoid allowing the solution to swirl, which will cause the cells to accumulate in the center of the dish and result in uneven cell adhesion. For best results, use a volume that does not result in a meniscus, which can also affect cell evenness upon seeding. (In our experiments a volume of 300-400  $\mu$ L was ideal.) To encourage even cell seeding, if your cell type allows, the dish can be kept on a warm surface (37°C) for the first 20 minutes to allow initial adhesion without disturbing the cell solution.

(5) Removing the unattached cells will reduce cell clustering and bridging between micropatterns.