



2D Cell Culture in PeptiGels®

This protocol describes the use of PeptiGels® for 2-dimensional (2D) cell culture.



We highly recommend the use of a **positive displacement pipette** (such as the Gilson piston pipette) to allow easy pipetting as these are viscous gels.

We recommend the use of cell inserts (such as the Greiner Bio-One cell inserts or equivalent) to increase gel stability and media diffusion.

As a guide, this protocol has been written for a total volume of 0.2mL PeptiGel®. Please scale up or down according to culture requirements.

2D Cell Culture Protocol

- Pre-wet the inserts in media/PBS for 1 hr to prevent bubbles getting trapped into the membrane pores.
- Remove PeptiGel® from the fridge and pre-warm to room temperature.
Hint: If required, centrifuge PeptiGel® for 1 min at 2500 g (3000 rpm) to remove air bubbles.
- Pipette a volume sufficient enough to cover the surface of the insert. As a guide, 0.2 mL PeptiGel® is enough for a 24 well plate.

Hint: Gently tap the plate against a sterile surface approximately 20 times to obtain a flat PeptiGel® surface.

Hint: If necessary, centrifuge the plate containing the insert for 1 min at 2500 g (3000 rpm).

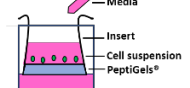
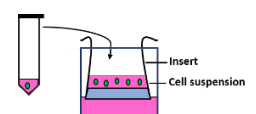
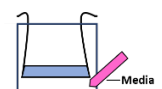
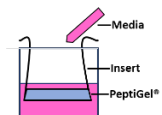
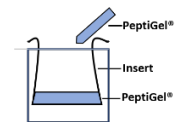
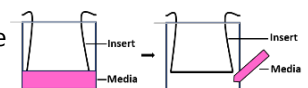
- Add 1 mL of culture media to the well containing the inserts and incubate at 37°C for 30 mins.
- Add 0.2 mL of culture media to the surface of pre-conditioned PeptiGel® in the insert and incubate at 37°C for 30 mins.

Hint: Pre-conditioning ensures the pH of PeptiGel® becomes neutral (pH 7).

Hint: Pre-conditioning also ensures a uniform distribution of media nutrients before cell seeding.

- Resuspend your cells to the required cell density in 0.2 mL of culture media.
- Remove the media used to pre-condition PeptiGel®.
Hint: Leave some media on the surface of pre-conditioned PeptiGel® to prevent the pipette tip disrupting the PeptiGel®.
- Transfer 0.2 mL of the resuspended cell suspension on top of the PeptiGel® and wait for 5 minutes
- Add 1 mL of fresh culture media to the plate and incubate overnight.

- Next day**, change the media and repeat as necessary depending on your cell requirements



Disclaimer

All standard safety procedures regarding cell culture need to be observed

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