PeptiGel® Protocol:

2D Cell Culture

Before you begin, please note:

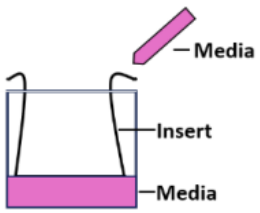
This protocol describes the use of PeptiGels® for 2-dimensional (2D) cell culture.

It's recommended to use a positive displacement pipette (such as the Gilson piston pipette) to allow easy pipetting as these are viscous hydrogels. Use of an air displacement pipette could lead to the introduction of bubbles to your PeptiGel®.

It's also recommended to use cell inserts (such as Greiner Bio-One Thincerts™ or equivalent) to increase gel stability and cell culture medium diffusion.

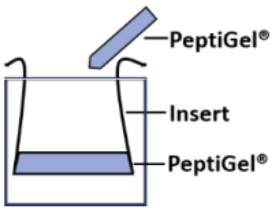
This protocol has been written for a total volume of 0.2 mL PeptiGel® with 24-well inserts as an example. Scale up or down according to culture requirements.

1. Pre-wet the inserts in cell culture medium/PBS for 1 hour to prevent bubbles getting trapped into the membrane pores.

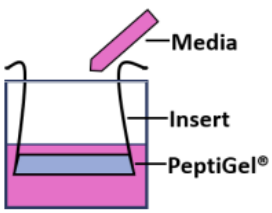


2. Remove PeptiGel® from the fridge and pre-warm to room temperature. If bubbles are present in the PeptiGel®, centrifuge the vial containing PeptiGel® at 1,600 x *g* for 1 minute, repeat if required.

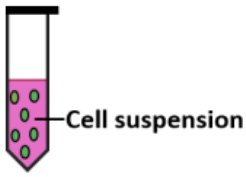
3. Pipette the PeptiGel® in sufficient quantity to cover the surface of the insert. In this example, 0.2 mL PeptiGel® is used for a 24-well plate insert. Gently tap the plate against a sterile surface to smooth the PeptiGel® surface.



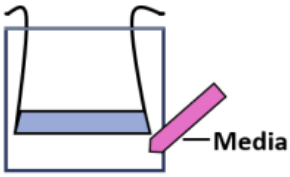
4. To pre-condition the PeptiGel®, add 1 mL of culture medium to the well containing the inserts and incubate at 37°C for 30 minutes. Then, carefully add 0.2 mL of the cell culture medium to the surface of the hydrogel and incubate for a further 30 minutes. Pre-conditioning ensures uniform distribution of nutrients and neutral pH.



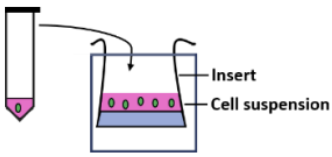
5. Resuspend your cells to the required cell density in 0.2 mL of culture medium.



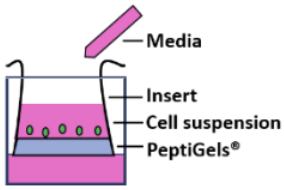
6. Remove the cell culture medium used to pre-condition the PeptiGel®, ensuring you leave some on the surface to prevent any disruption from the pipette tip.



7. Transfer 0.2 mL of the resuspended cell suspension on top of the pre-conditioned PeptiGel® and incubate at 37°C for 5 minutes.



8. Add 1 mL of fresh cell culture medium around the insert and incubate at 37°C overnight.



9. After **24 hours**, replace the cell culture medium. Repeat the cell culture medium replacement depending on the requirement of your cell type.

For further support, please contact our technical support team at tech@cellgs.com.

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