PeptiGel®

Redefining Cell Culture for Life Science

APPLETON WOODS

3D Cell Culture in PeptiGels®

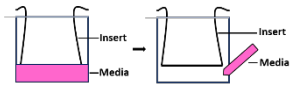
This protocol describes the use of PeptiGels® for 3-dimensional (3D) cell culture.

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.

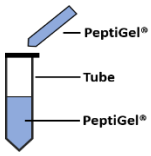
We recommend the use of cell inserts (such as the Greiner Bio-One cell inserts or equivalent) to increase gel stability and media diffusion.

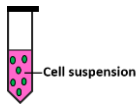
As a guide, this protocol has been written for a total volume of 1mL PeptiGel®. Please scale up or down according to culture requirements.

3D Cell Culture Protocol

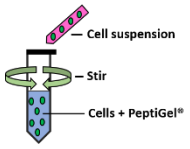


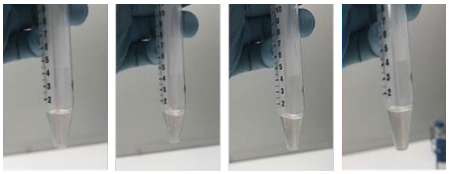
- Pre-wet the inserts in media/PBS for 1 hr to prevent bubbles getting trapped into the membranes pores.
- Remove PeptiGel® from the fridge and pre-warm to room temperature.
  Hint: *If required, centrifuge PeptiGel® for 1 min at 2500 g (3000 rpm) to remove air bubbles.*
- Pipette 1 mL of PeptiGel® into a 15 mL Falcon tube.



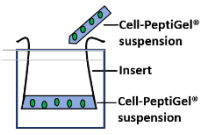


- Resuspend your cells in up to 200 µL of culture media.
  Hint: Please note, a more concentrated cell suspension is required to achieve the target cell densities as your cell suspension will be diluted in 1 mL of PeptiGel®.
- Transfer the cell suspension to the bottom of the tube containing 1 mL of PeptiGel® and carefully mix the two together.
  Hint: *To ensure a homogenous mixture, insert the pipette to the bottom of the gel and release the cells slowly whilst gradually bringing the pipette upwards, in a stirring motion.*

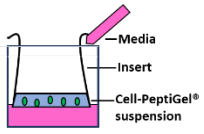




  Hint: *To avoid bubbles, make sure the pipette tip never leaves the hydrogel while mixing. Please note, there are useful supporting videos on our website (link – on new website)*



- Pipette aliquots of gel-cell mixture into the inserts.
  Hint: *As a guide, dispense 50 – 100 µL into an insert depending on the desired thickness according to pipette supplier instruction.*



- Add approximately 600 µL of media to the bottom of each well.

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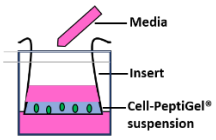
- Put the plate in an incubator for 5 mins.
  Hint: *If there are bubbles in the media, seal the plate and agitate by tapping on the bench carefully.*
- Add 250 µL of media carefully to the top of inserts and put the plate in an incubator.
  Hint: *To avoid any evaporation boundary effect, add PBS or water to any empty well.*
- Change the media on top and bottom of the inserts 2-3 times within 1 hr to ensure homogenous diffusion of media nutrients.
  Hint: *Leave some media on the surface of the gel to prevent the pipette tip disrupting the gel.*
- 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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