PeptiGel® Protocol:

How to Coat a Well Plate

Before you begin, please note:

This protocol describes the use of PeptiGels® Alpha 1™, Alpha 2™ and Alpha 4™ for coating culture plates to grow cells.

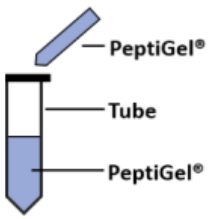
It's recommended to use a positive displacement pipette (such as the Gilson piston pipette) to allow easy pipetting as these are viscous hydrogels. Use of an air displacement pipette could lead to the introduction of bubbles to the PeptiGel®.

The thickness of the coating can influence cell behaviour, hence the optimal thickness is dependent on the experimental plan and intended outcome.

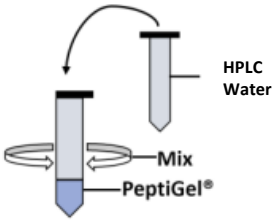
This protocol outlines how to coat one well of a 24 well plate with a 1 mm thick layer. Scale up or down according to your culture requirements.

The volume used may be adjusted based on your desired thickness, and the surface area of the plate used. For example, adding 200 µL of gel to a well in a 24 well plate with a diameter of 15.6 mm and surface area of 1.9 cm^2, will give you a thickness of 1.05 mm.

1. 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.



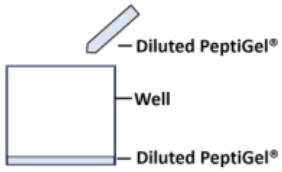
2. Using a positive displacement pipette, transfer 0.75 mL of PeptiGel® into a tube.



3. Add 0.25 mL of HPLC-grade water to the PeptiGel® and vortex for 3 minutes.

4. Centrifuge the vial containing PeptiGel® at 1,600 x *g* for 1 minute, repeat if required.

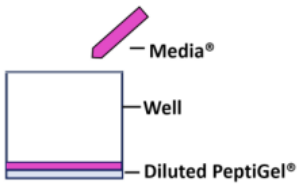
5. Add 0.2 mL of diluted PeptiGel® to one well in a 24-well plate.



6. Gently swirl the plate to ensure an even coverage of the surface of the well. Gently tap the plate against a sterile environment 15 times to aid flattening of the surface of the PeptiGel®.

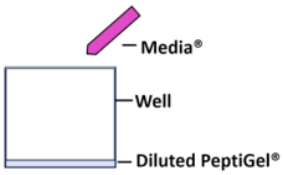
   Optional step: Centrifuge the plate for 1 minute at 1,600 x *g* for 1 minute to obtain a flat surface of PeptiGel®.

7. Carefully add 1 mL of cell culture medium to cover the surface of the PeptiGel® coating. Add the culture medium by pipetting slowly and gently down the wall of the well to avoid disturbance of the coating.



8. Incubate at room temperature (~25°C) for 30 minutes for the PeptiGel® to gelate. Thicker coatings may require longer incubation times.

9. Remove the cell culture medium from the top of the PeptiGel®.



10. Continue your experiment as required. If the plate is not required immediately, seal with sealing film and store at 4°C until required.

    Note: PeptiGels® have a 12-month shelf life once opened in a sterile environment.

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

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