

# PROTOCOL

## Petaka coating with Matrigel

1 Thaw frozen Matrigel leaving it at 4°C overnight.



2 Prepare 10 Petakas ready to be used with a couple of stands.



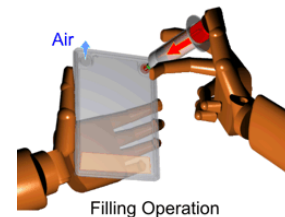
3 Prepare a sterile 30 mL syringe, and Celartia's tips.



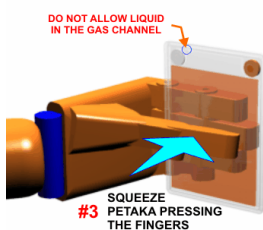
4 Prepare 20 ml of Diluted Matrigel (1 mg/ml) in serum-free cold cell culture media (RPMI1640, EMEM, DMEM, or others).



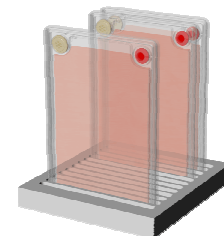
5 Introduce the 20 mL of diluted Matrigel into a Petaka.



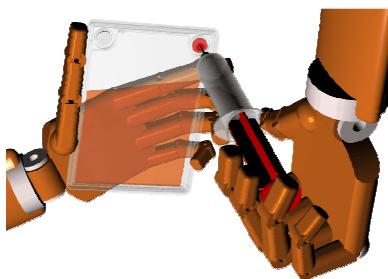
6 Squeeze the Petaka until the media reaches the upper limit of the Petaka chamber, and hold for 10 seconds, then release.



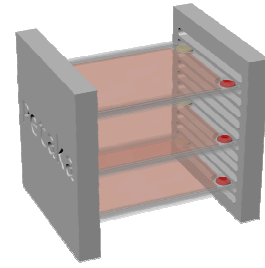
7 Leave Petaka in a vertical position for 5 minutes.



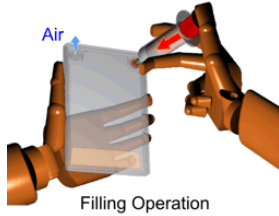
8 Slowly withdraw the Matrigel solution from Petaka.



- 9 Put the Petaka in a completely horizontal position whilst all Petakas are being prepared



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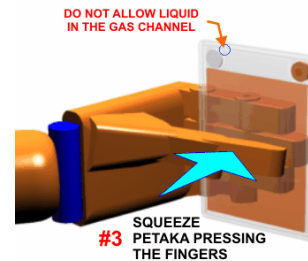


Introduce the residual Matrigel solution into the next Petaka.

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Squeeze the new petaka until the media reaches the upper limit of the petaka chamber, and hold 10 seconds.

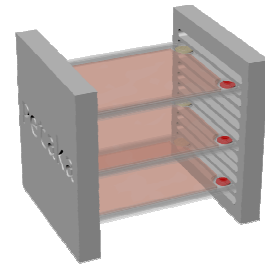
**Repeat the steps 6 to 10 for the remaining Petakas**



Notice that each Petaka treated will reduce the volume of Matrigel solution by around 2 to 3 mL. Therefore it is possible to treat efficiently about 5 Petakas covering about 80% of the available culture surface of the Petaka's chamber, which will lead to a residual 5 to 10 mL of Matrigel solution that could be saved at  $-80^{\circ}\text{C}$ , until it is reused.

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Incubate Petakas, in a horizontal position, at  $37^{\circ}\text{C}$  for 6 hours before seeding cells



Phase contrast microscopy of cells inside Petaka cultured on Matrigel

