PeptiGel® Protocol:

How to Extract Organoids from PeptiGel® cultures

Before you begin, please note:

This protocol describes how to extract organoids cultures in PeptiGels® for further analysis.

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®.

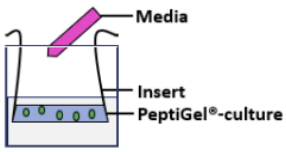
This protocol has been written as a guide. Therefore, further assay optimization may need to be carried out for your cultures.

Before using this protocol, ensure that the cell density of the culture is high (>60% confluency).

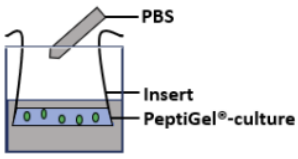
Requirements:
1. Cell Recovery Solution
2. BSA in PBS (1% wt/vol)

1. It is recommened to use this protocol with PeptiGels® made using the 3D Cell Culture method.

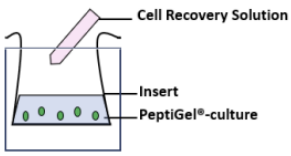
2. Carefully remove the cell culture medium from the PeptiGel® culture.



3. Wash three times with PBS for 5 minutes adding sufficient PBS to fully cover the PeptiGel®. To minimize disturbance to the PeptiGel®, leave some PBS on the PeptiGel® surface after each wash step.

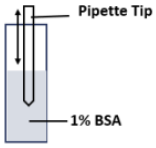


4. Remove the PBS and add 1 mL of ice-cold Cell Recovery Solution to the well containing the PeptiGel® cultures.

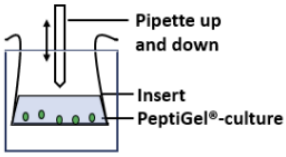


5. Leave the gels to incubate with the Cell Recovery Solution for 1-2 hours at 4°C. To increase the rate of digestion, place on a rocker with gentle agitation.

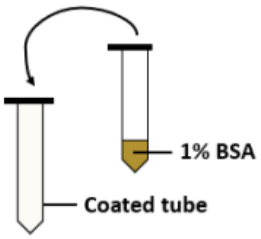
6. Meanwhile, pre-coat 1 mL tip with 1% BSA in PBS by pipetting up and down twice. This will prevent the organoids sticking to the plastic.



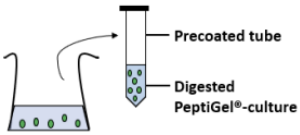
7. Following the incubation, resuspend your organoids in the culture by pipetting up and down 10-15 times.



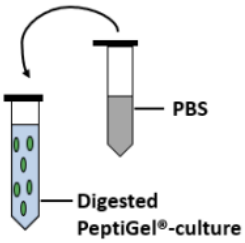
8. Pre-coat a 15 mL Falcon tube with 1% BSA in PBS by adding 1-2 mL and then discard.



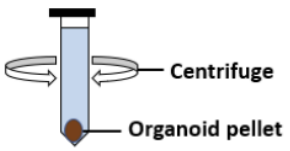
9. Transfer the digested culture into the pre-coated 15 mL Falcon tube. To remove the remaining organoids in the culture, rinse the now empty well with 1 mL of 1% BSA and add into pre-coated tube.



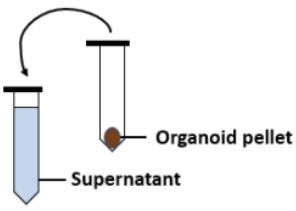
10. Add PBS to the digested cultures in the 15 mL Falcon tube to achieve a final volume of 10 mL.



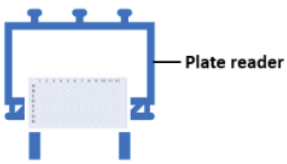
11. Centrifuge the mixture at 800 rpm (70 g) for 3 minutes at 4°C to pellet the organoids.



12. Discard the supernatant, leaving the pellet containing the organoids in the tube.



13. The remaining organoids are now ready for further sub-cultures and/or analysis.



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