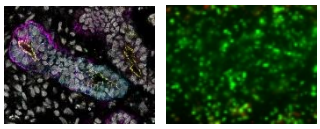
PROTOCOL

How to extract Organoids from PeptiGel® Cultures



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

General advice

We highly recommend the use of a **positive displacement pipette** (such as the Gilson piston pipette) to minimise bubble formation during pipetting as these are viscous gels.

Please note, this protocol has been written as a guide only. Further assay optimisation may be needed for your cultures.

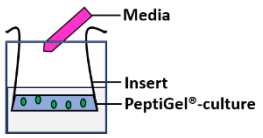
Please ensure that the cell density of your cultures is high (> 60% confluency) before using this protocol.

Requirements and equipment

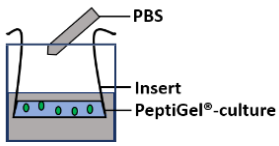
- Cell Recovery Solution (Corning – product code 354253)
- BSA in PBS (1% wt/vol)

Protein Extraction Protocol

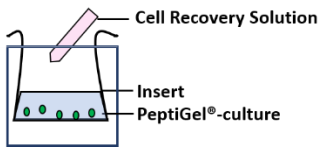
- Carefully remove the cell culture media from the PeptiGel® culture.
  Hint: Be careful not to touch the gel with the pipette tip while aspirating.



- Wash with PBS for at least 5 - 10 minutes and repeat once or twice.

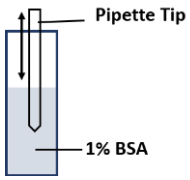


- Remove the PBS and add 1 mL of ice-cold Cell Recovery Solution to the well containing PeptiGel® cultures.



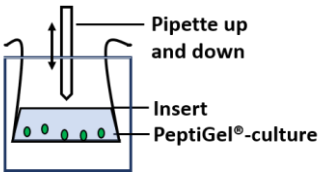
- Leave the gels to incubate with the Cell Recovery Solution for 1 – 2 hours at 4°C.
  Hint: Put on a rocker with gentle agitation to increase the rate of digestion.

- Pre-coat 1mL tip with 1% BSA in PBS by pipetting up and down twice.
  Hint: Pre-coating with protein solution prevents organoids sticking to the plastic.

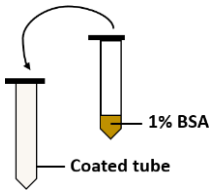


Alderley Park | Cheshire | SK10 4TG
t: +44 (0) 1625 238800
e: info@manchesterbiogel.com
w: www.manchesterbiogel.com

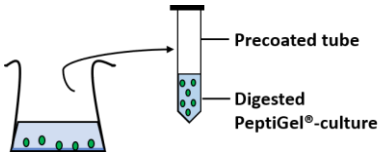
- Following the incubation, resuspend your organoids in the culture by pipetting up and down 10 – 15 times.
  Hint: Pipette gently to prevent the disruption of the organoid structures.



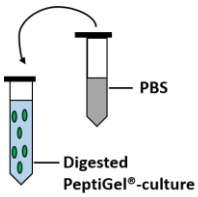
- Pre-coat a 15mL tube with 1% BSA in PBS by adding 1 -2 mL into the tube and then discard.
  Hint: Pre-coating with protein solution prevents organoids sticking to the plastic.



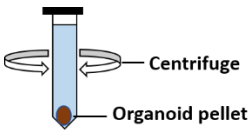
- Transfer the digested cultures into the pre-coated 15ml falcon tube
  Hint: To remove the remaining organoids in the culture, rinse the now empty well with 1mL of 1% BSA and add into the pre-coated tube.



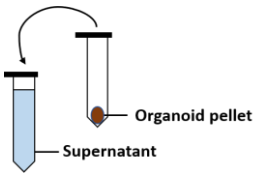
- Add PBS to the digested cultures in the 15mL falcon tube to achieve a final volume of 10 mL.



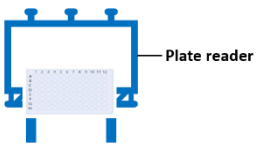
- Centrifuge the mixture at 800 rpm (70 g) for 3 minutes at 4°C to pellet the organoids.



- Discard the supernatant and leave the pellet (containing the organoids).
  Hint: Discard as much supernatant as possible while leaving enough to cover the pellet to make sure the organoids are not disrupted.



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



Support

For further support, please contact our technical support team on +44 (0) 1625 238 800 or info@manchesterbiogel.com. Please note there are also useful supporting videos on our website (www.manchesterbiogel.com/resources/technicalvideos).

Disclaimer

All standard safety procedures regarding cell culture need to be observed.