PeptiGel® Protocol:

Sample Preparation for Protein Extraction from PeptiGel® cultures

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Before you begin, please note:

This protocol describes protein extraction from cells cultured in PeptiGel ®.

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.

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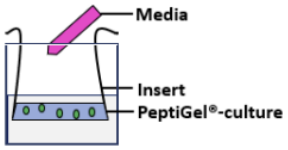
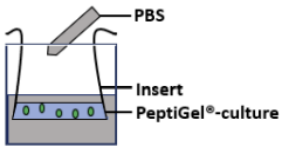
1. It is recommended to use this protocol with PeptiGels® made using the 3D Cell Culture method.

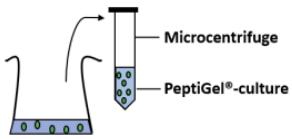
2. Carefully remove the cell culture medium from the PeptiGel® culture.



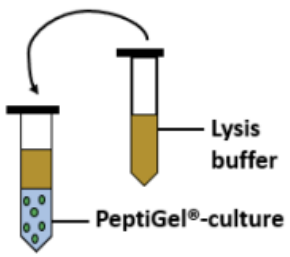
3. Wash three times with PBS for 5 minutes adding sufficient PBS to fully cover the PeptiGel®. To minimize disturbance to the PeptiGel®, leave some PBS on the PeptiGel® surface after each wash step.



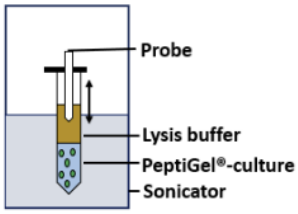
4. Remove the PBS then remove the bottom membrane from the insert and transfer the PeptiGel® to a microcentrifuge tube.



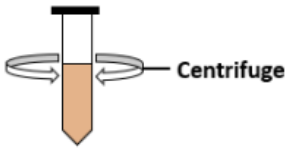
5. Add 100 µL of RIPA or Urea lysis buffer to digest the PeptiGel® culture.



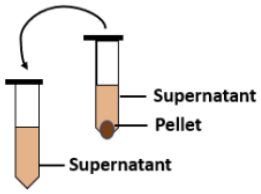
6. Homogenise, or sonicate, samples until complete dissolution is observed.



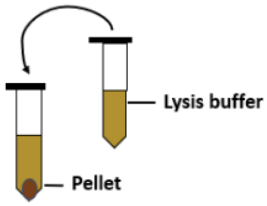
7. Centrifuge the mixture at 5,000 rpm (2,800 *g*) for 5 minutes at 4°C.



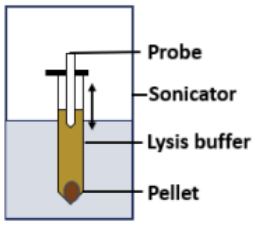
8. Collect the supernatant into a fresh Eppendorf tube.



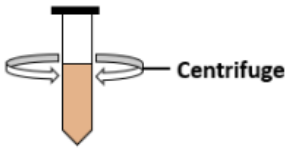
9. Add 100 µL of lysis buffer to the pellet.



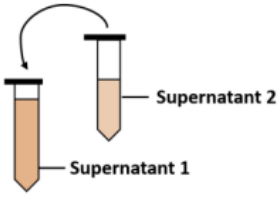
10. Homogenise, or sonicate, the sample until complete dissolution is observed.



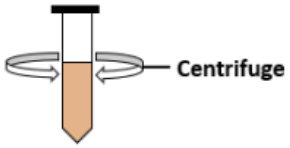
11. Centrifuge the mixture at 5,000 rpm (2,800 *g*) for 5 minutes at 4°C.



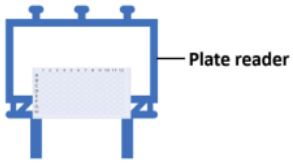
12. Collect the supernatant and combine with the previous supernatant.



13. Centrifuge at a maximum speed of 13,000 rpm for 3 minutes.



14. Measure the total protein concentration using any of the standard assays, such as BCA or Bradford assays, or equivalent.



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

General info@cellgs.com
Technical Enquiries tech@cellgs.com
Orders order@cellgs.com

www.cellgs.com

EUROPE
Cell Guidance Systems Ltd
Maia Building
Babraham Bioscience Campus
Cambridge
CB22 3AT
United Kingdom
T +44 (0) 1223 967316
F +44 (0) 1223 750186

USA
Cell Guidance Systems LLC
Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA
T 760 450 4304
F 314 485 5424