PeptiGel® Protocol:

Sample Preparation for RNA Extraction from PeptiGel® cultures

---

Before you begin, please note:

This protocol describes RNA extraction from cells cultures 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®.

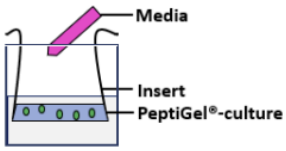
It's also recommended to use a column-based approach relying on RNA-specific binding to a silica membrane under conditions of high ionic strength such as the RNeasy Mini Kit from Qiagen.

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

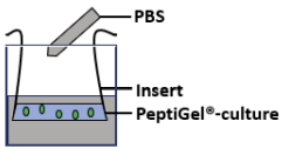
---

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

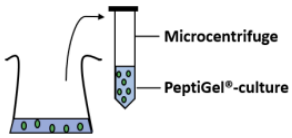
2. Carefully remove the cell culture medium from the PeptiGel® culture.



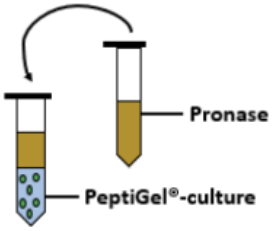
3. Wash three times with PBS for 5 minutes adding sufficient PBS to fully cover the PeptiGel®. To minimize disturbance to the PeptiGel®, leave some PBS on the PeptiGel® surface after each wash step.



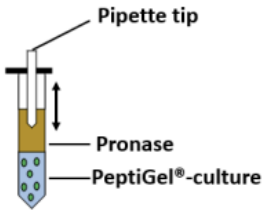
4. Remove the PBS then remove the bottom membrane from the insert and transfer the PeptiGel® to a microcentrifuge tube.



5. Add pronase solution (10 mg/mL stock solution) to the PeptiGel® culture. The volume should match the volume of PeptiGel® used. E.g. for 200 µL of PeptiGel®, use 200 µL pronase solution.

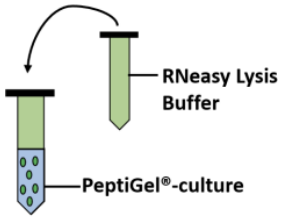


6. Carefully pipette up and down to dissolve the hydrogel.

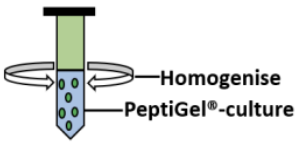


7. Incubate for 5 minutes at 37°C.

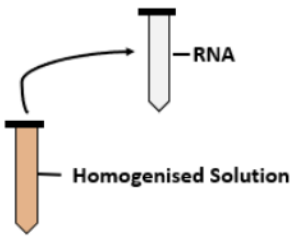
8. To initiate RNA extraction from the PeptiGel® culture add 350 µL of RNeasy lysis buffer, or equivalent, if using a different RNA extraction kit.



9. Homogenise the lysate by centrifugation at a maximum speed of 10,000 rpm (13,000 *g*) for 3 minutes, or equivalent if using a homogeniser.



10. Extract the RNA according to the guidelines given by the manufacturer or specific kit being used.



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