Promega

Product Application

Total RNA isolation from 3D cell cultures or cells in Matrigel® with the ReliaPrep™ miRNA Tissue Kit.

To isolate total RNA, including micro RNAs from cells in 3D culture.

Kit: ReliaPrep™ miRNA Tissue Kit (Cat. #Z6210)

Analyses: RT-qPCR

Sample Type(s): Cells grown in microspheres or cells in Matrigel®.

Materials Required:

- Microsphere plate, hanging drop culture, or cell basement membrane such as Corning® Matrigel®

This protocol was developed by Promega Applications Scientists and is intended for research use only.

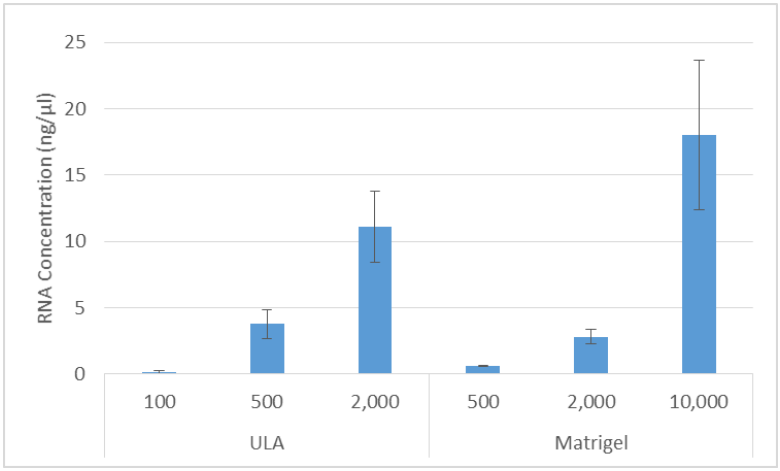
Users are responsible for determining suitability of the protocol for their application.

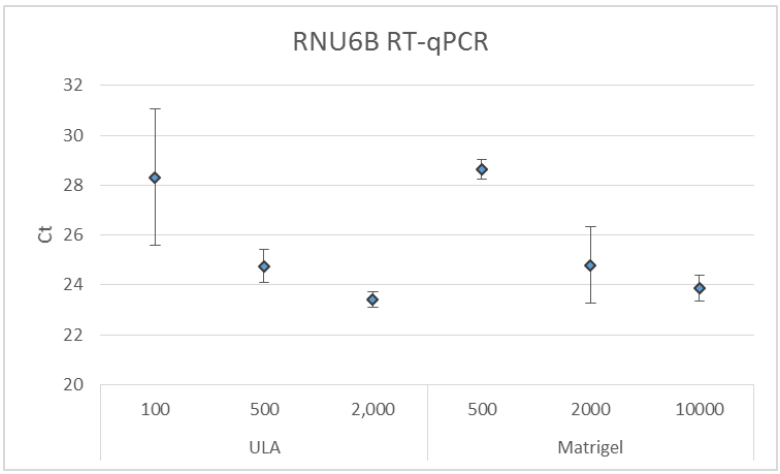
Further information can be found in Technical Manual #TM469, available at: **www.promega.com/protocols** or for further information, please contact **techserv@promega.com**

Protocol:

1. Prepare microspheres or cells in Matrigel® and grow until RNA isolation is desired.
2. Prepare LBA +TG, RWA, and DNase as indicated in TM469.
3. Carefully remove media from microtissue or Matrigel®.
4. Add 200µl of LBA +TG to each well. Pipet gently 7-10 times with a p1000 pipet to homogenize. Remove liquid from well into a 1.5ml microcentrifuge tube. NOTE: For cells in Matrigel®, wait 30 seconds to allow matrix to dissolve prior to gently pipetting to homogenize.
5. Add 130µl of RDB and vortex for 10 seconds.
6. Centrifuge at 12,000 X *g* for 2 minutes.
7. Transfer homogenate to a new 1.5ml tube.
8. Add 400µl of 100% isopropanol to each cleared homogenate. Mix by vortexing.
9. Transfer homogenate to a ReliaPrep™ Minicolumn. Centrifuge at 12,000 X *g* for 30 seconds.
10. Discard the liquid in collection tube.
11. Add 500µl RWA to each column. Centrifuge at 12,000 X *g* for 30 seconds. Discard liquid in collection tube.
12. Add 500µl RWA to each column. Centrifuge at 12,000 X *g* for 2 minutes.
13. Transfer column to a 1.5ml Elution Tube.
14. Add 40µl of Nuclease-Free Water to each column. Centrifuge at 12,000 X *g* for 1 minute.
15. Transfer 5µl of DNase I and 5µl DNase 10X Buffer to each eluate.
16. Incubate 5 minutes at room temperature.
17. Add 150µl LBA to the samples.
18. Add 300µl of 95% ethanol to mixture and vortex for 10 seconds. Transfer mixture to a new ReliaPrep™ Minicolumn.
19. Centrifuge 12,000 X *g* for 30 seconds. Discard the liquid in the collection tube.
20. Add 500µl RWA. Centrifuge12,000 X *g* for 30 seconds. Discard liquid in the collection tube.
21. Add 500µl RWA. Centrifuge 12,000 X *g* for 2 minutes. Discard liquid in the collection tube.
22. Transfer column to a 1.5ml Elution Tube.
23. Add 30µl Nuclease-Free Water. Centrifuge 12,000 X *g* for 1 minute. If expected yields are greater than 15µg, add an additional 15µl of Nuclease-Free water and repeat the centrifugation step.

Results:





RNA isolation from HCT116 cells grown four days in Corning® ULA plates or Corning® Matrigel® Basement Membrane Matrix using the ReliaPrep™ miRNA Tissue Kit. The x-axis of each graph is labelled with growing condition (Matrigel or ULA plate) and cell number plated in a 96-well plate (100-10,000). RNA concentration was measured using the QuantiFluor® RNA System on the Quantus™ Fluorometer (Top). RT-qPCR was performed with miRNA control gene RNU6B to determine amplifiability of RNA samples and presence of miRNA (Bottom). Shown are the averages ± std for an n=2 for each condition.

