Total RNA Isolation from 3D Cell Cultures or Cells in Matrigel® Matrix Using the ReliaPrep™ miRNA Cell and Tissue Miniprep System

Isolate total RNA, including microRNA from cells in 3D culture.

Promega Corporation

Sample Type: Cells grown in microspheres or in Corning Matrigel® matrix.

Material Required: Microsphere plate, hanging drop culture or cell basement membrane such as Corning Matrigel® matrix.

Analysis: RT-qPCR

Protocol: ReliaPrep™ miRNA Cell and Tissue Miniprep System Technical Manual #TM469.

Disclaimers:
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 e-mail: **techserv@promega.com**

Protocol

1. Prepare microspheres or cells in Matrigel® matrix 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® matrix.
4. Add 200µl of LBA +TG to each well. Pipet gently 7–10 times with a p1000 pipet to homogenize. Remove liquid from well and transfer into a 1.5ml microcentrifuge tube.

   Note: For cells in Matrigel® matrix, 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 × *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 × *g* for 30 seconds.
10. Discard the liquid in the collection tube.
11. Add 500µl of RWA to each column. Centrifuge at 12,000 × *g* for 30 seconds. Discard liquid in collection tube.
12. Add 500µl of RWA to each column. Centrifuge at 12,000 × *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 × *g* for 1 minute.
15. Transfer 5µl of DNase I and 5µl of DNase 10X Buffer to each eluate.
16. Incubate 5 minutes at room temperature.
17. Add 150µl of LBA to the samples.
18. Add 300µl of 95% ethanol to the mixture and vortex for 10 seconds. Transfer mixture to a new ReliaPrep™ Minicolumn.

19. Centrifuge at 12,000 × *g* for 30 seconds. Discard the liquid in the collection tube.
20. Add 500µl of RWA. Centrifuge at 12,000 × *g* for 30 seconds. Discard liquid in the collection tube.
21. Add 500µl of RWA. Centrifuge at 12,000 × *g* for 2 minutes. Discard liquid in the collection tube.
22. Transfer column to a 1.5ml Elution Tube.
23. Add 30µl of Nuclease-Free Water. Centrifuge at 12,000 × *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

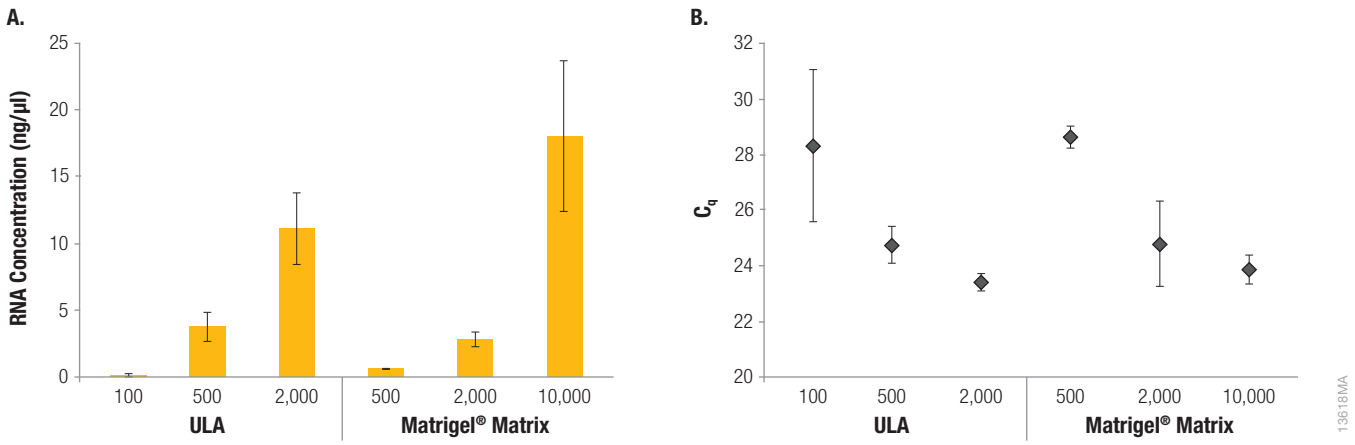

Figure 1. RNA isolation from HCT116 cells grown four days in Corning ULA plates or Corning Matrigel® basement membrane matrix using the ReliaPrep™ miRNA Cell and Tissue Miniprep System. The X axis of each graph is labelled with growing condition (ULA plate or Matrigel® matrix) and cell number plated in a 96-well plate (100–10,000). **Panel A.** RNA concentration was measured using the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer (Cat.# E6150). **Panel B.** RT-qPCR was performed using the TaqMan® miRNA reverse transcription kit and GoTaq® Probe qPCR Master Mix (Cat.# A6102) targeting the small RNA control gene RNU6B to determine amplifiability of RNA samples and presence of miRNA. Shown are the averages ± standard deviation for an n=2 for each condition.

Ordering Information

Product	Size	Cat.#
ReliaPrep™ miRNA Cell and Tissue Miniprep System	10 preps	Z6210
	50 preps	Z6211
	250 preps	Z6212

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