Promega

Product Application

Automated Purification of Total RNA from Spheroids Grown in Ultra-Low Attachment Plates

Purify total RNA including microRNAs from cells grown in ultra-low attachment plates to form spheroids using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)

Analyses:

- UV absorbance
- Dye-based quantitation
- RT-qPCR
- TapeStation analysis

Sample Type(s): Cells grown in 96-well ultra-low attachment (ULA) plates

Input: Spheroids prepared with 500-10,000 cells

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)
- 96-well Spheroid Microplates (Corning, Cat.# 4520)

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.

For further information, see Technical Manual TM441, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Grow cells in ULA plates to form spheroids until RNA purification is desired.
2. Carefully remove growth medium from each spheroid.
3. Add 200µl of 1-Thioglycerol/Homogenization solution, prepared as stated in the Maxwell® RSC miRNA Tissue Kit Technical Manual (TM441), directly to each well.
4. Pipette up and down to break up spheroid. Transfer contents to 1.5ml tube.
5. Add 200µl of Lysis Buffer, 200µl of Lytic Enhancer, and 30µl of Proteinase K to the sample in 1-Thioglycerol/Homogenization Solution.
6. Vortex for 20 seconds.
7. Incubate at room temperature for 10 minutes.
8. Transfer 630µl of lysate to well #1 of the Maxwell® RSC miRNA Tissue Kit Cartridge.
9. Add 10µl of blue DNase I Solution, prepared as stated in the technical manual, to well #4 of the Maxwell® RSC Cartridge.
10. Add a plunger to well #8.
11. Add elution tubes with 50µl of Nuclease-Free Water.
12. Run the miRNA Tissue protocol on the Maxwell® RSC Instrument.

Results:

Total RNA including miRNA was successfully purified from 500, 2,000, 5,000, and 10,000 cells grown in ULA plates to form spheroids using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument. RNA was of high quality, and three miRNAs and one mRNA target were successfully amplified.

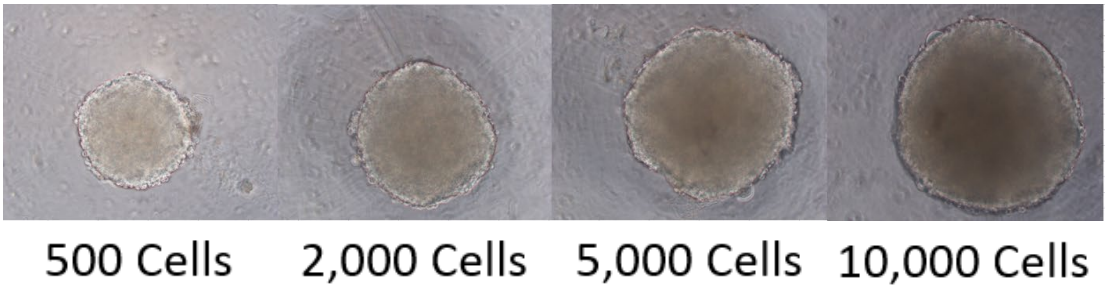

Figure 1. Images of cells grown in ULA plates to form spheroids. HCT116 cells were plated in a titration of 500, 2,000, 5,000, or 10,000 cells per well. Images were taken with 100X magnification after four days of growth at 37°C 5% CO_2.

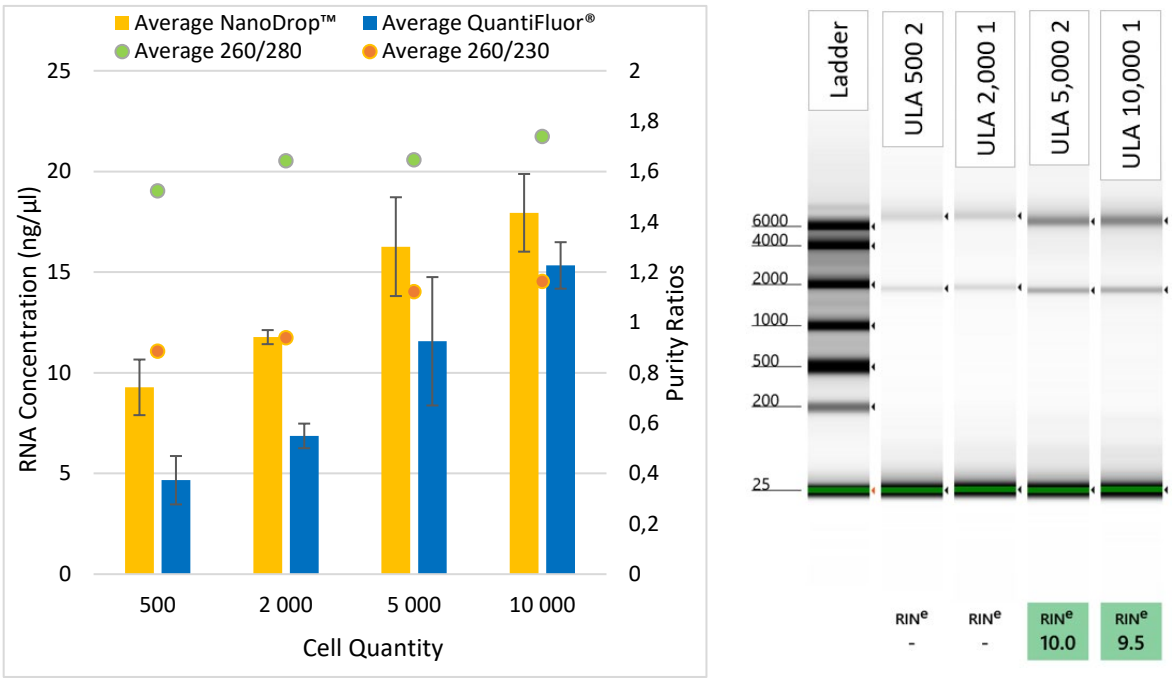

Figure 2. RNA eluate quantitation results. Total RNA including miRNA was purified from cells grown in ULA plates to form spheroids using the Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460). **Left:** The RNA concentration of eluates was measured using a NanoDrop™ 8000 spectrophotometer and the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer. Data represent the average concentration and standard deviation of triplicate purifications. **Right:** The eluate from each cell count with the highest concentration was selected for TapeStation RNA ScreenTape electrophoresis. The concentrations of the 500 and 2,000 cell samples were outside of the detectable range used to calculate a RIN^e score.

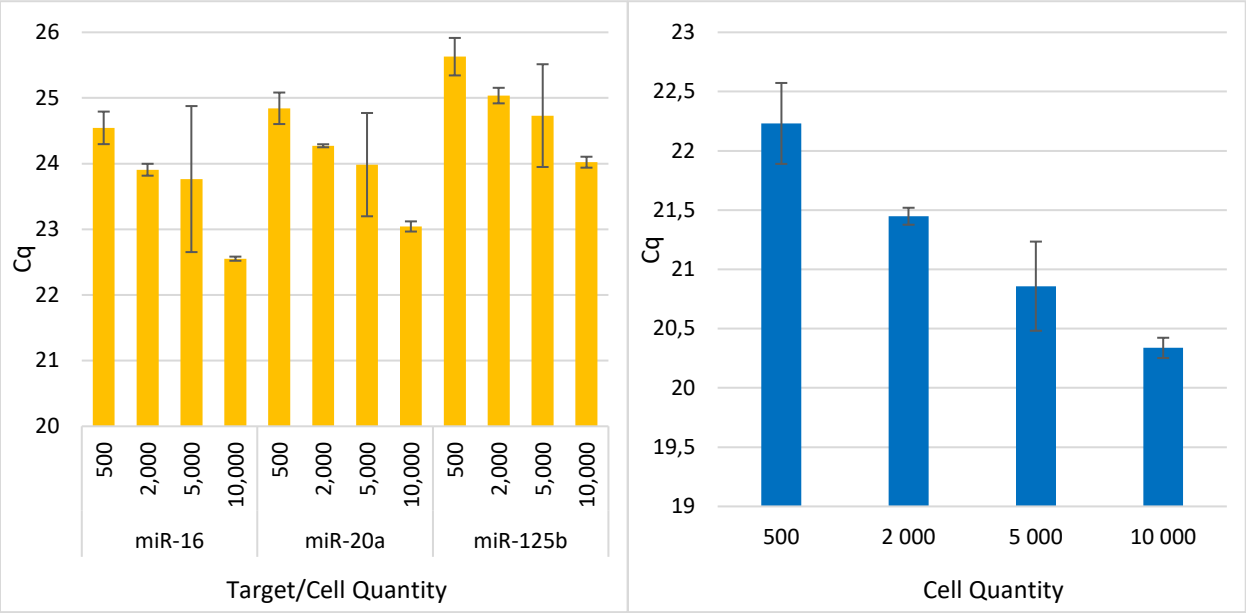

Figure 3. RT-qPCR amplification results. Triplicate purifications of 500, 2,000, 5,000, or 10,000 cells grown in ULA plates to form spheroids were completed using the Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460). **Left:** miRNAs were detected using TaqMan™ MicroRNA Reverse Transcription Kit (Cat.# 4366597, Thermo Scientific) with TaqMan™ assays for miR-16, miR-20a, and miR-125b. **Right:** β2M RNA was detected using GoTaq® 1-Step RT-qPCR System (Cat.# A6020). Data represent the average Cq value and standard deviation of triplicate purifications amplified in duplicate.