Promega

Product Application

Automated Purification of Total RNA from Cells Grown in Matrigel®

Purify total RNA including microRNAs from cells grown in Matrigel®.

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

Analyses:

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

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

Input: Microtissues grown from 2,000-5,000 cells

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Matrigel® Matrix (Corning®, Cat.# 354234)

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

Results:

Total RNA including miRNA was successfully purified from cells grown in Matrigel® using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument. Total RNA including miRNA was purified from a titration of 2,000 and 5,000 cells.

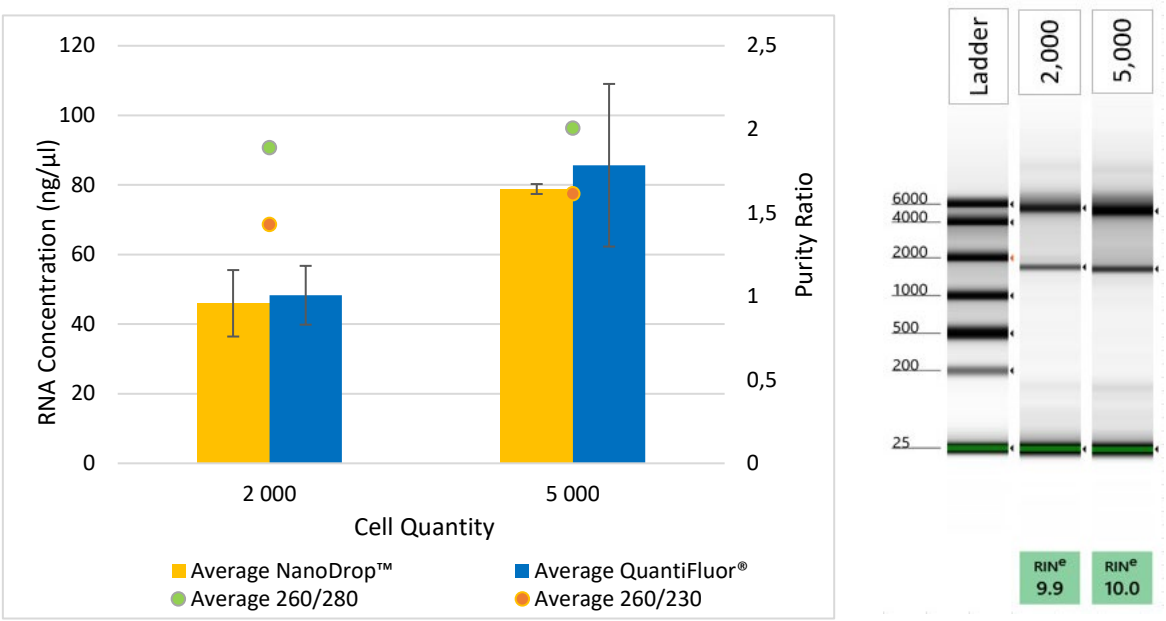

Figure 1. RNA eluate concentration results. Total RNA including miRNA was purified from HCT116 cells grown in Matrigel® using the Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460). The RNA concentration of eluates was measured using a NanoDrop™ 8000 spectrophotometer and the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer. Data represent the average concentration and standard deviation of triplicate purifications.

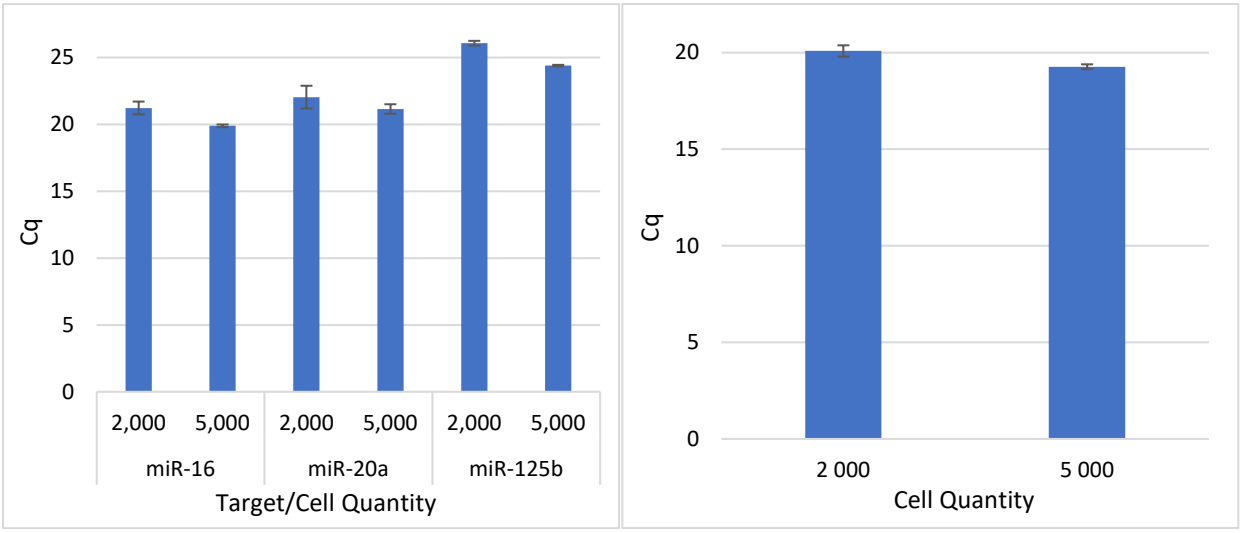

Figure 2. RT-qPCR amplification results. Triplicate purifications of 2,000 or 5,000 HCT116 cells grown in Matrigel® were completed using the Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460). **Left:** miRNAs were detected using TaqMan™ MicroRNA Reverse Transcription Kit (Cat.# AS1460, Thermo Scientific) 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.