Promega

Product Application

Automated Purification of Total RNA from TRIzol™ Samples

Purify total RNA, including miRNA from TRIzol™ samples with Maxwell® RSC Instrument and the Maxwell® RSC miRNA Plasma and Serum Kit.

Kit: Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)

Analyses: RT-qPCR, UV absorbance

Input: RNA samples in TRIzol™

Materials Required:

- Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Vortex
- Heat block

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 TM546, available at: **www.promega.com/protocols**

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

Protocol:

Total RNA including miRNA can be extracted from a TRIzol™ lysate or from the aqueous phase of a TRIzol™ extraction. Omit Steps 2-4 if a TRIzol™ lysate is used for purification. Note, using a TRIzol™ lysate results in lower total RNA recovery compared to an equivalent volume of TRIzol™ aqueous phase sample.

1. Lyse and homogenize samples in TRIzol™ reagent then incubate for 5 minutes.
2. Add 0.2ml chloroform per 1ml TRIzol™ reagent used for lysis. Incubate 2-3 minutes.
3. Centrifuge the samples for 15 minutes at 12,000 x *g* at 4°C.
4. Transfer the aqueous phase containing the RNA to a new tube.
5. Add 200µl of TRIzol™ sample (lysate or aqueous layer) to a microfuge tube.
6. Add 80µl Proteinase K and 230µl of Lysis Buffer C. Mix by vortexing for 5 seconds.
7. Incubate at 37°C for 15 minutes. During this time prepare Maxwell® RSC Cartridges as described in the Maxwell® RSC miRNA Plasma and Serum Kit Technical Manual (TM546, Section 5.C).
8. Transfer the entire sample to well #1 of the Maxwell® RSC Cartridge.
9. Add 10µl of blue DNase I Solution (TM546 Section 4.A) to well #4 of the Maxwell® RSC Cartridge. The reagent in well #4 should turn green.
10. Proceed to Section 6 of TM546 for loading samples onto the instrument.

Results:

200µl of a TRIzol™ lysate or 200µl of a TRIzol™ aqueous phase sample from 500,000 K562 cells was extracted using the Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680). As a comparison, 200µl of a TRIzol™ aqueous phase sample was extracted manually following the TRIzol™ Reagent User Guide. Total RNA including miRNA can be extracted from a TRIzol™ lysate or from the aqueous phase of a TRIzol™ extraction using the Maxwell® RSC miRNA Plasma and Serum Kit. Using a TRIzol™ lysate yields less total RNA compared to a TRIzol™ aqueous phase sample.

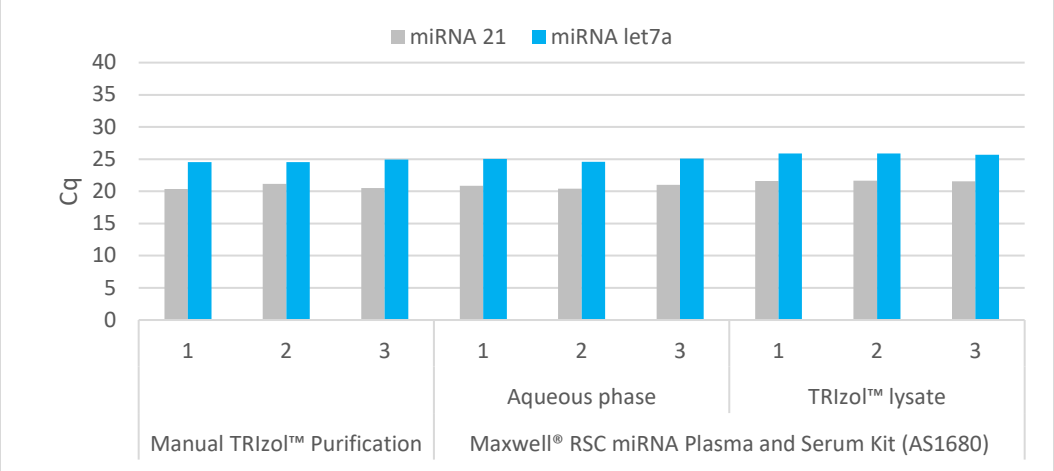

Figure 1. Average TaqMan® miRNA Cq results (n=2). TaqMan® assays for miRNA-21 and let7a were amplified with GoTaq® Probe qPCR Master Mix (Cat.# A6101) using 1.3µl of cDNA prepared using the TaqMan® miRNA RT Kit (Applied Biosystems) with 5µl of purification eluate. Equivalent miRNA Cqs were observed with both Maxwell® extractions and the manual TRIzol™ extraction.

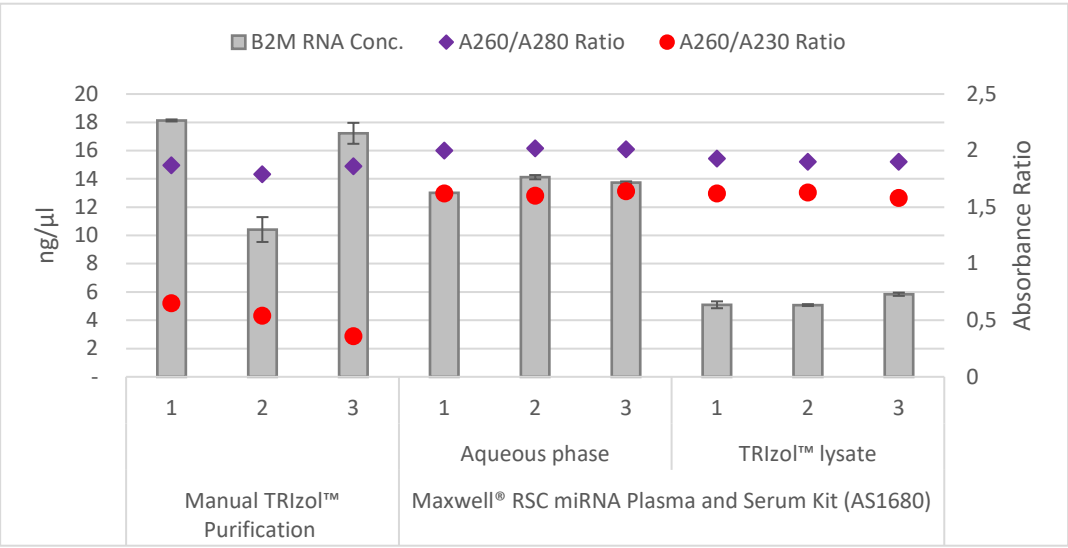

Figure 2. Total RNA concentration was determined using RNA specific primers to β-2-microglobulin using GoTaq® Probe 1-Step RT-qPCR system (Cat.# A6120). Increased total RNA was observed with the TRIzol™ aqueous phase sample compared to the TRIzol™ lysate sample. Improved A260/A230 ratios were observed with the Maxwell® extractions compared to the manual TRIzol™ extraction. N=2.