

Total RNA including miRNA extraction from TRIzol™ samples using ReliaPrep™ miRNA Cell and Tissue Miniprep System

Extraction of total RNA including miRNA from TRIzol™ samples using the miRNA Cell and Tissue Miniprep System.

Kit: ReliaPrep™ miRNA Cell and Tissue Miniprep System (Cat. #Z6211)

Analyses: 1-step RT-qPCR, 2-step RT-qPCR using TaqMan® miRNA assays, NanoDrop™

Input: RNA samples in TRIzol™

Materials Required:

- Benchtop Centrifuge
- 100% Isopropanol
- Vortex

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 by e-mailing 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 higher 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. Follow TM469 starting at Step 5 in **Section 5. RNA Isolation and Purification from Cell Samples.**

Results:

200µl of a TRIzol™ lysate or 200µl of a TRIzol™ aqueous phase sample from 500,000 K562 cells was extracted using ReliaPrep™ miRNA Cell and Tissue MiniPrep System (Z6211). Samples were eluted in 30µl.

Both miRNA and total RNA can be extracted from a TRIzol™ lysate or from the aqueous phase of a TRIzol™ extraction using the ReliaPrep™ miRNA Cell and Tissue MiniPrep System. Using a TRIzol™ lysate results in higher total RNA recovery compared to an equivalent volume of TRIzol™ aqueous phase sample.

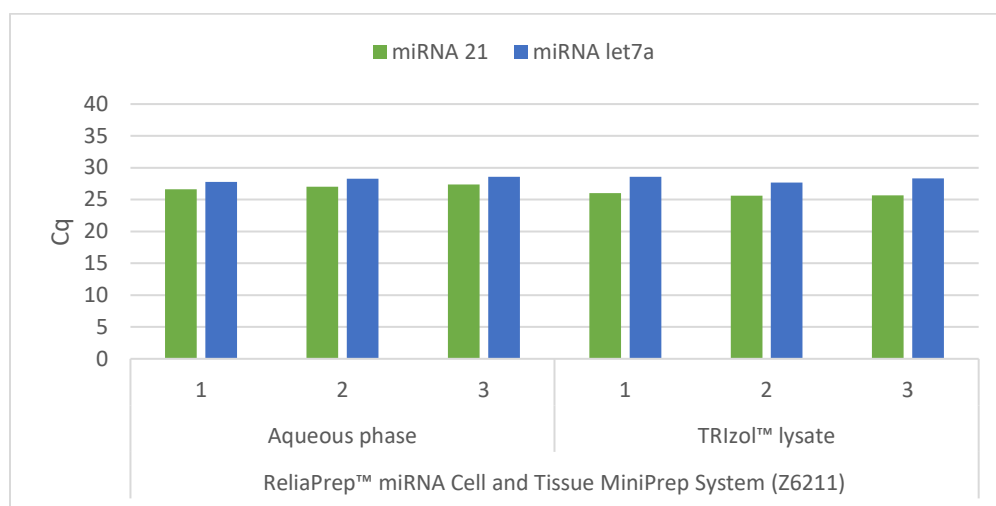


Figure 1. Average TaqMan® miRNA Cq results (n=2). Equivalent miRNA Cqs were observed with ReliaPrep™ extractions from both the aqueous phase and TRIzol™ lysate.

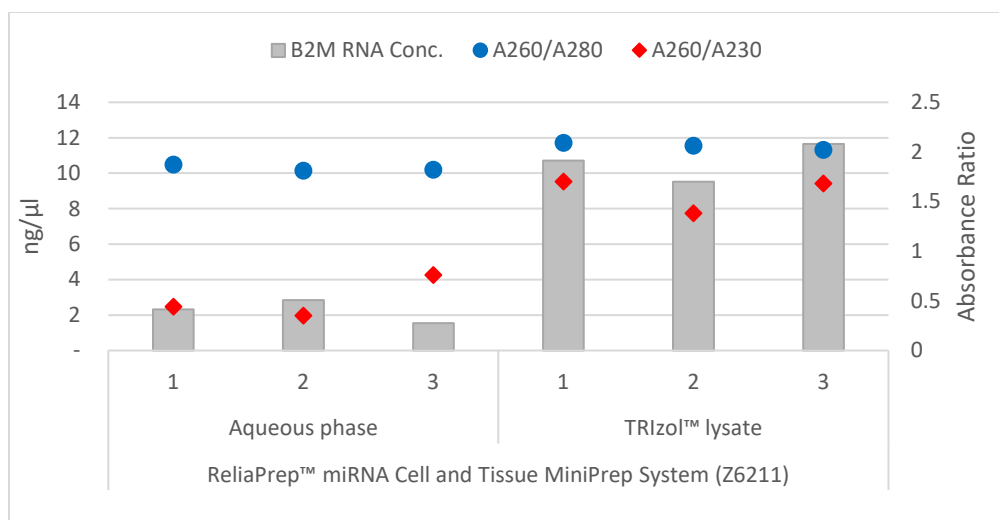


Figure 2. Total RNA concentration was determined using RNA specific primers to β-2-microglobulin (n=2). Reduced total RNA was observed with the TRIzol™ aqueous phase sample compared to the TRIzol™ lysate sample. Improved A260/A230 ratios were observed with the TRIzol™ lysate sample compared to the TRIzol™ aqueous phase sample.