Promega

Product Application

RNA purification from the aqueous phase following TRIzol™/ chloroform extraction using ReliaPrep® RNA clean up and Concentration System

RNA was successfully purified from the aqueous phase of TRIzol™/ Chloroform extracted samples using ReliaPrep® RNA Clean-Up and Concentration System

Kit: ReliaPrep® RNA Clean-Up and Concentration System (Cat. #Z1071)

Analyses: UV absorbance with NanoDrop™ One
Fluorescence-based quantitation with QuantiFluor® RNA System
TapeStation analysis of RNA integrity
mRNA and miRNA amplification by RT-qPCR

Sample Type(s): 3T3 cells in culture

Input: approx. $0.7x10^6$ cells

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 #TM541, available at: www.promega.com/protocols

or by e-mailing technical services at techserv@promega.com

Materials Required:

- ReliaPrep® RNA clean up and Concentration System (Cat. #Z1071)
- TRIzol™ reagent (Cat.# 15596026), Invitrogen
- Chloroform

Protocol:

1. Culture mammalian cells in recommended medium.
2. Remove medium. (Do not wash cells with PBS to avoid RNA degradation.)
3. Add 1ml of TRIzol™ Reagent per $75cm^2$ flask (approx. $2x10^6$ cells), scrape the cells into the TRIzol™ Reagent, pipet lysate up and down and transfer the cells to a 1.5ml tube.
4. Incubate on ice for 5 minutes.
5. Add 0.6ml of chloroform per ml of TRIzol™ Reagent.
6. Incubate sample for 2 minutes.
7. Centrifuge sample for 15 minutes at 12,000x g at 4°C.
8. Transfer the aqueous phase to a new 1.5ml tube.
9. Purify RNA from the aqueous phase using the ReliaPrep® RNA Clean-Up and Concentration System following recommendation in the technical manual (TM541).

Results:

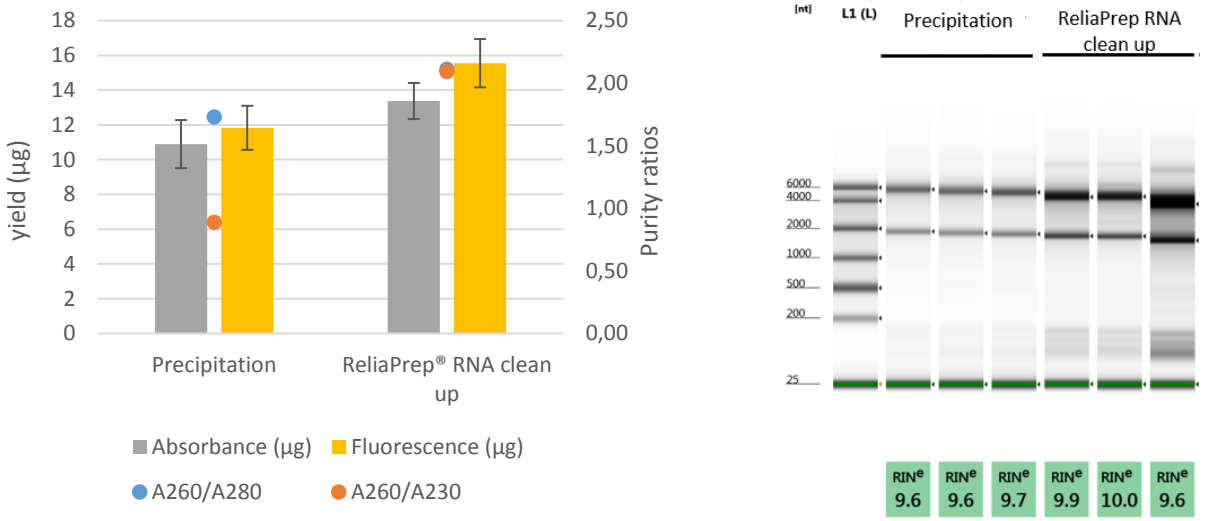

Figure 1. RNA yield, purity and integrity obtained from 3T3 cells. Left. RNA (from approx. 0.7 x 10^6 cells) was purified from the aqueous phase of TRIzol™/ Chloroform extracted samples using the ReliaPrep® RNA Clean-Up and Concentration System (elution in 15µl) and compared with the classical precipitation method (elution in 20µl). Concentration and purity were measured by UV absorbance using NanoDrop™ One and fluorescent-dye-based method using QuantiFluor® RNA System. N=3. Error bars represent the standard deviation of the mean for triplicate purifications. **Right**. TapeStation analysis of purified RNA using an RNA ScreenTape.

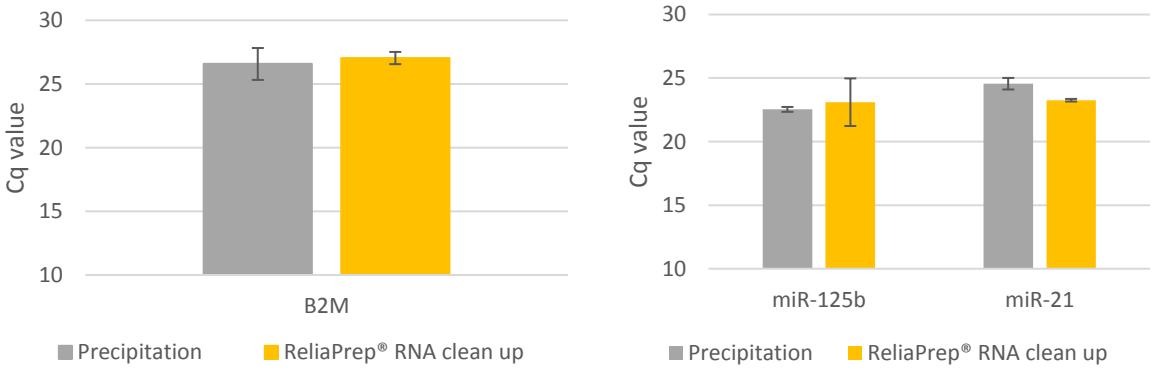

Figure 2: mRNA and miRNA amplification. Left. Level of mRNA were compared by amplification of 25ng of RNA using GoTaq® 1-step RT-qPCR Master Mix and B2M mRNA specific primers. N=3. Error bars represent the standard deviation of the mean for triplicate purifications. **Right.** Level of miRNA were compared by amplification of 10ng of RNA using TaqMan Micro RNA assay for miR21 and miR125b using TaqMan™ MicroRNA Reverse Transcription Kit and GoTaq® Probe qPCR Master Mix. N=3. Error bars represent the standard deviation of the mean for triplicate purifications.