Promega

Product Application

Automated Purification of RNA from TRIzol™ Samples Prepared from Whole Blood

Purify RNA from aqueous layer and direct TRIzol™ lysates of white blood cells from up to 20ml of human whole blood using the Maxwell® RSC Instrument and Maxwell® RSC miRNA Plasma and Serum Kit

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

Analyses: UV absorbance, dye-based quantitation and TapeStation

Sample Type(s): TRIzol™ lysate and aqueous layer of TRIzol™ lysate from 20ml of Human Whole Blood

Input: up to 650µl

Materials:

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

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

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

Protocol:

TRIzol™ lysate and aqueous layer preparation

1. Add up to 20ml of whole blood to a conical tube.
2. Add the recommended volume of differential lysis solution to lyse the red blood cells while leaving the white blood cells intact.
3. Centrifuge to pellet the white blood cells.
4. Remove the supernatant and resuspend the cell pellet in TRIzol™. Mix well by pipetting/vortexing.
5. Proceed to RNA purification (see below), or add 200µl of chloroform to 1ml of TRIzol™ sample. Vortex well.
6. Incubate at room temperature for 2 minutes.
7. Centrifuge at 12,000 x *g* for 15 minutes at 4°C.
8. Remove the colorless upper aqueous phase containing the RNA (~650µl).
9. Proceed to RNA purification (see below).

RNA purification

1. Add up to 650µl of TRIzol™ aqueous layer or direct TRIzol™ lysate to a 1.5ml microtube.
2. Add 230µl of Lysis Buffer C and mix by vortexing for 5 seconds.
3. Optional: Add 80µl of Proteinase K and mix by vortexing for 5 seconds. Incubate at 37°C for 15 minutes.
4. Add entire sample to well #1 of the RSC cartridge.
5. Set up cartridges according to the Maxwell® RSC miRNA Plasma and Serum Kit Technical Manual (TM546) and run the miRNA Plasma and Serum method on the Maxwell® RSC Instrument.

Results:

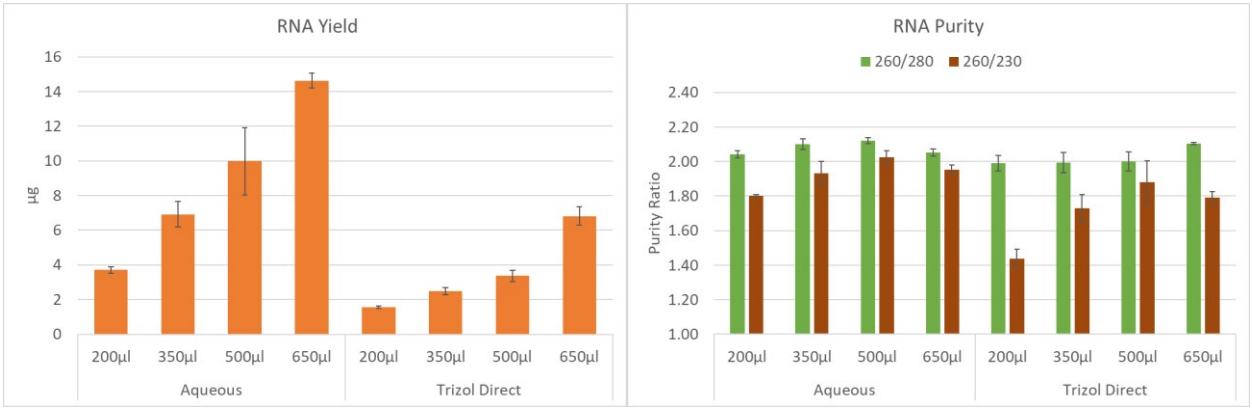

Figure 1. Yield (left) and purity (right) of RNA purified from TRIzol™ aqueous layer and direct TRIzol™ lysate made from whole blood using the Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680). Yield based on the QuantiFluor® RNA System (Cat.# E3310). Purity based on NanoDrop™ (Thermo Fisher Scientific). Volumes added for each purification are given. Data represent the mean of N=3 replicates.

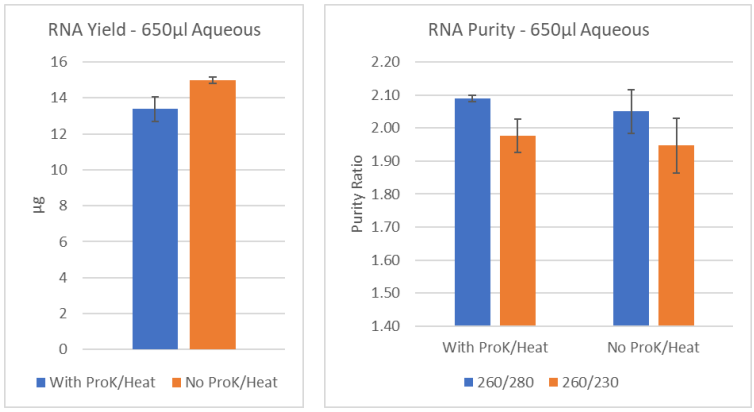

Figure 2. Yield (left) and purity (right) of RNA purified from 650µl TRIzol™ aqueous layers. Yield based on the QuantiFluor® RNA System (Cat.# E3310). Purity based on NanoDrop™ (Thermo Fisher Scientific). Samples were processed with and without a heated Proteinase K incubation. Data represent the mean of N=3 replicates.

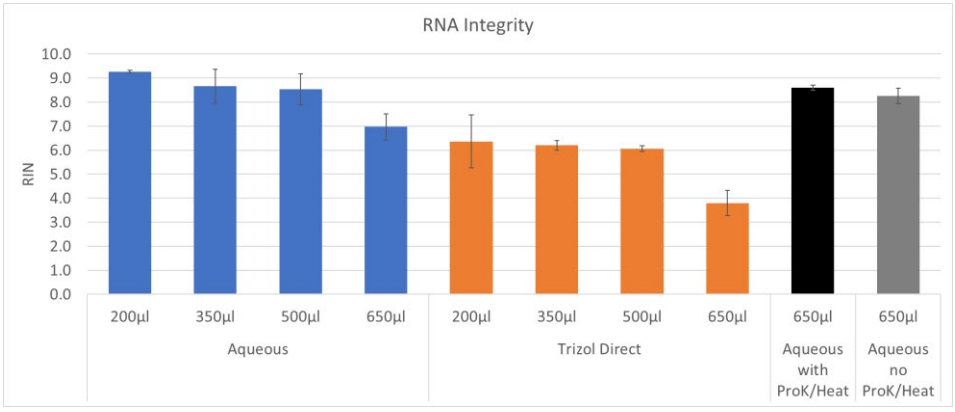

Figure 3. Average RIN values of RNA purified from various volumes of TRIzol™ aqueous layers and direct TRIzol™ lysates based on 4200 TapeStation System (Agilent). 650µl samples were also processed with and without a heated Proteinase K incubation. RNA ScreenTape was used. Data represent the mean of N=3 replicates.