Promega

Product Application

Automated purification of Total RNA from Large Volumes of Plasma

Purify total RNA including miRNA from large volumes of plasma with the Maxwell® RSC instrument and Maxwell® RSC miRNA Plasma kit

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

Analyses: RT-qPCR and Dye-based quantitation

Samples: Plasma or serum

Input: 4ml of plasma or serum

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 and AX1115 Custom Protocol, available at: **www.promega.com/protocols**

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

Materials Required:

- Maxwell® RSC miRNA Plasma and Serum kit (Cat.# AS1680)
- Maxwell® RSC instrument (Cat.# AS4500)
- rotisserie shaker
- heat block, 37°C
- 100% Isopropanol (~200ml for 48 × 4ml samples)
- additional Binding Buffer C, Custom (89ml additional for 48 × 4ml samples. Cat.# AX573A (20ml) or AX301B (200ml)
- Additional Proteinase K (27ml additional for 48 × 4ml samples; Cat.# MC5008 (16ml) or A5051 (23ml)

Protocol:

Reconstitute DNase I by adding 275µl of Nuclease-Free Water to the vial of DNase. Invert or swirl to mix.

1. Transfer 4ml of plasma or serum to a 50ml conical tube.
2. Add 640µl of Proteinase K.
3. Add 1.84ml of Binding Buffer and vortex for 30 seconds.
4. Incubate sample at 37°C for 15 minutes with no agitation.
5. Pipet to resuspend resin in well #2 of Maxwell® Cartridge, making sure resin is completely resuspended before transferring it to the 50ml containing sample.
6. Add 3.6ml of 100% Isopropanol.
7. Incubate for 45 minutes while rotating or shaking. The resin must be kept in suspension for the entire incubation.
8. Centrifuge tubes at 1,000 × *g* for 2 minutes to pellet the resin.
9. Place a magnet alongside resin pellet to fix it in place (i.e. Z5410 magnetic stand). With tube on

stand, carefully remove as much supernatant as possible with a pipette. **Note:** Some foam is expected and will be eliminated with the addition in Step 10.

10. Using a pipette, transfer the contents of well #1 into the tube containing the magnetic resin.
11. Resuspend the resin by pipetting. Do not vortex, as this may result in resin adherence to the sides of the tube.
12. Transfer the resuspended resin back to well #1 of the Maxwell® cartridge.
13. Add 230µl of Binding Buffer and 200µl of Nuclease-Free Water to well #1.
14. Place a plunger in well #8 of cartridge and add 50–100µl of Nuclease-Free Water to each elution tube.
15. Add 10µl of resuspended DNase I to well #4 (yellow).
16. Run on Maxwell® RSC instrument with the RSC miRNA Tissue protocol.

Results:

Isolation of total RNA from plasma was performed as described above and compared to the standard 500µl input method. RNA quantitation was performed with dye-based quantitation using QuantiFluor® RNA System (Cat.# E3310) on a Quantus™ Fluorometer (Figure 1). 2-step RT-qPCR analysis using GoTaq® Probe qPCR Master mix (Cat.# A610A) was used to detect miR-16 target.

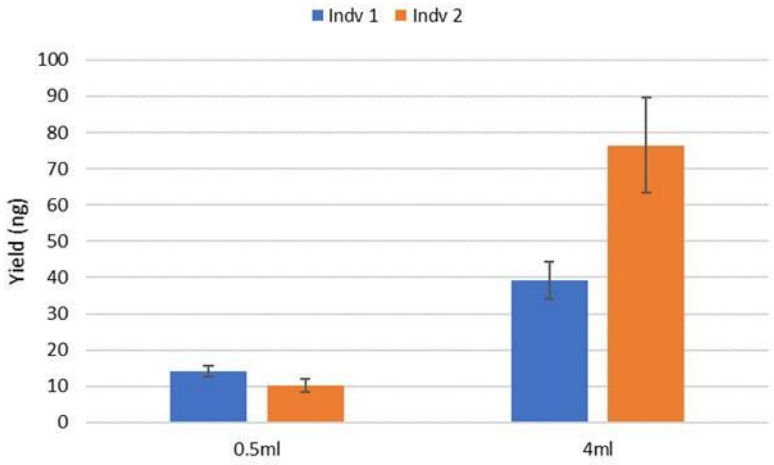

Figure 1. RNA yield measured by dye-based quantitation using QuantiFluor® RNA System (Cat.# E3310).

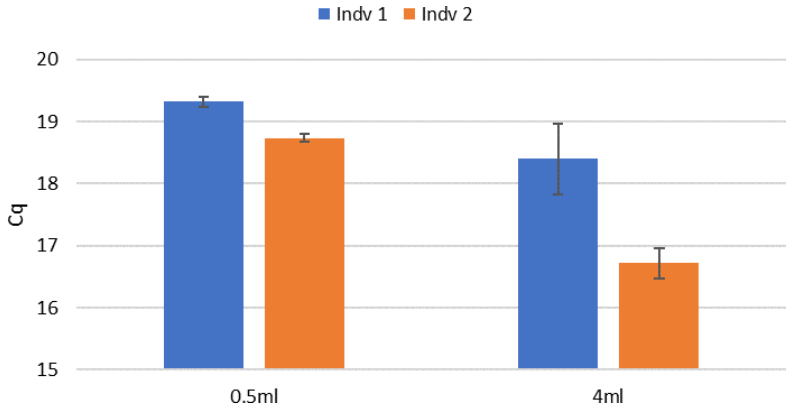

Figure 2. Cq values from RT-qPCR to detect miR-16 with RNA isolated from plasma.