Isolating Total RNA from Large Volumes of Plasma Using the Maxwell® RSC miRNA Plasma and Serum Kit

Promega Corporation.

Kit:
Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)
Sufficient for 48 automated isolations from plasma, serum or enriched exosome samples. Cartridges are single-use only.
Includes:

- Lysis Buffer C, 25ml
- Proteinase K (PK) Solution, 5 vials
- DNase I (lyophilized), 2 vials
- Blue Dye, 50µl
- Maxwell® RSC Cartridges, 48
- Maxwell® RSC Plunger Pack, 48 Plungers
- Elution Tubes (0.5ml), 50 Tubes
- Nuclease-Free Water, 25ml

Analyses:
GoTaq® RT-qPCR System (Cat.# A6020 or Cat.# 6010) and QuantiFluor® RNA System (Cat.# E3310)

Input:
Plasma or Serum (4ml)

Materials Required:

- Rotisserie Shaker
- Heat Block, 37°C
- PolyATract® System 1000 Magnetic Separation Stand (Cat.# AS1240) OR MagneSphere® Magnetic Technology Stand (Cat.# Z5332 or Cat.# Z5342)
- Conical tubes, 50ml
- Aerosol-resistant micropipette tips
- 100% Isopropyl Alcohol (~200ml for 48 × 4ml Samples)
- Binding Buffer C, Custom
  (Additional buffer may be needed; 89ml for 48 × 4ml Samples)
  - Cat.# AX573A (20ml) or Cat.# AX301B (200ml)
- Proteinase K
  (Additional Proteinase K may be needed; 27ml for 48 × 4ml Samples)
  - Cat.# MC5008 (16ml) or Cat.# A5051 (23ml)

Introduction

MicroRNAs (miRNAs) are endogenous small non-coding RNAs 18-24 nucleotides in size that play important roles involving gene regulation associated with cancer, disease control and gene silencing. Due to the impact on disease progression, miRNA research is rapidly shifting towards biomarker discovery.

Many of the commercially available miRNA purification methods involve organic extraction during preprocessing. Here, we describe the use of the Maxwell® RSC miRNA Plasma and Serum Kit to extract RNA purification, including smaller RNA, such as miRNA, from large volumes of plasma. This chemistry offers significant advantages with a simple automated workflow, no organic extraction and minimal preprocessing.

Protocol

This protocol was developed by Promega Applications Scientists and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application.

1. Reconstitute DNase I by adding 275µl of Nuclease-Free Water to the vial of DNase. Invert or swirl the vial to mix.
2. Transfer 4ml of Plasma or Serum sample to a 50ml conical tube.
3. Add 640µl of Proteinase K to each tube containing Plasma or Serum sample.
4. Add 1.84ml of Binding Buffer to the sample tubes and vortex for 30 seconds.
5. Incubate sample tubes at 37°C for 15 minutes with no agitation.
6. Pipette to resuspend the resin in well #2 of the Maxwell® Cartridge. Make sure the resin is completely resuspended before transferring it to the sample tubes.
7. Add 3.6ml of 100% Isopropyl alcohol to the sample tubes.
8. Incubate the sample tubes for 45 minutes while rotating or shaking. The resin must be kept in suspension for the entire incubation.
9. Centrifuge tubes at 1,000 × *g* for 2 minutes to pellet the resin.
10. Place a magnet alongside the resin pellet to fix it into place (i.e., PolyATtract® System 1000 Magnetic Separation Stand, Cat.# Z5410). With the tube on the 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 11.

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

Results

Isolation of total RNA from the plasma of two donors was performed as described in the protocol steps above and compared to the standard 500µl input method. RNA quantitation was performed with dye-based quantitation using QuantiFluor® RNA System on a Quantus™ Fluorometer (Figure 1). GoTaq® RT-qPCR System with hsa-miR-16 primers (Thermo Fisher Scientific Assay ID 000391) was used to detect miR-16.

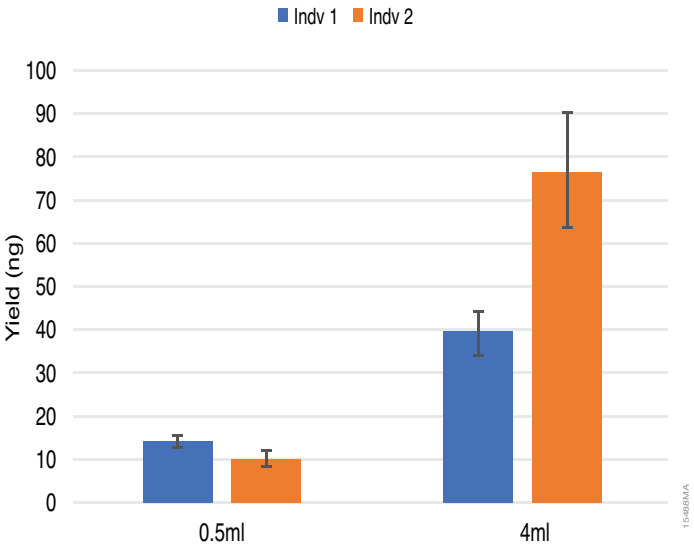

Figure 1. RNA yield measured by dye-based quantitation using QuantiFluor® RNA System.

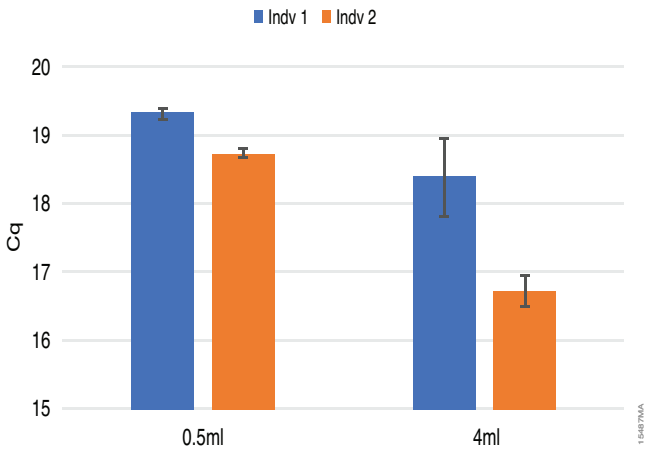

Figure 2. C_q values from GoTaq® RT-qPCR System to detect miR-16 with RNA isolated from plasma (n=3).