Promega

Product Application

Automated Purification of Total RNA from Urine

Purify total RNA including miRNAs from urine with the Maxwell® RSC Instrument and the Maxwell® RSC miRNA Plasma and Serum Kit.

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

Analyses: RT-qPCR

Sample Type: Urine

Input: 1ml - Whole urine, Cell-free urine, Urine cell pellet

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, 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) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- GoTaq® Probe qPCR Master Mix (Cat.# A6101)
- Urine collection containers
- Centrifuge
- Heat block for 1.5ml tubes at 37°C

Protocol (cell-free urine):

- If using whole urine (unspun) for purification, then begin at Step 7 with 1ml of whole urine.
- If only cell-free urine is of interest, whole urine can be filtered with a 1.6µm GF/A glass microfiber syringe filter to remove cells. Then begin at Step 7 with 1ml of cell-free urine.

1. Collect urine in desired urine collection containers.
2. Transfer urine into a conical tube and chill on ice for at least 5 minutes.
3. Centrifuge urine sample at 2,000 x *g* for 10 minutes at 4°C.
4. Transfer supernatant into a new tube. This is 1x cleared urine. The cell pellet may be saved for purification as well.
5. Centrifuge 1x cleared urine at 2,000 x *g* for 10 minutes at 4°C.
6. Transfer supernatant into a new tube. This is 2x cleared urine.
   a. The cell pellet may be combined with the pellet from Step 4 and used for purification. The combined cell pellet may be frozen at -80°C for later purification.
   b. If purifying the same day, add 100µl of Nuclease-Free Water to the pellet and begin at Step 8.
7. Add 1ml of 2x cleared urine into a 1.5ml tube.
8. Add 230µl of Lysis Buffer C and 80µl of Proteinase K. Mix by vortexing for 5 seconds.
9. Incubate at 37°C for 15 minutes. During this incubation, prepare Maxwell® RSC cartridges:
   a. Place Maxwell® RSC cartridges into the Maxwell® RSC deck tray. Remove seals.
   b. Add 10µl of reconstituted DNase I with blue dye to well #4.
   c. Add plungers to well #8.
   d. Add 50µl of Nuclease-Free Water to elution tubes and place in the deck tray.
10. Add the contents of the 1.5ml tube to well #1.
11. Run the miRNA Plasma and Serum Kit protocol on the Maxwell® RSC Instrument.
12. Store eluted RNA in aliquots at -20°C or below. Avoid repeated freeze-thaw cycles.

Results:

Urine was collected in sterile containers from 2 individuals or 3 individuals on separate days. Urine was processed into fractions as described above. Two *H. sapiens* miRNAs, let-7a and miR-16, were measured by RT-qPCR with the TaqMan™ MicroRNA Reverse Transcription Kit (ThermoFisher), using TaqMan™ assays 000377 (let-7a) and 000391 (miR-16) with 5µl of total RNA. Probe-based qPCR was performed with 1.3µl of cDNA using GoTaq® Probe qPCR Master Mix (Cat.# A6101) and the corresponding TaqMan™ primer mixes. miRNAs were successfully purified from whole urine and from the two urine fractions tested. Results were similar between cell-free urine prepared by centrifugation versus filtration.

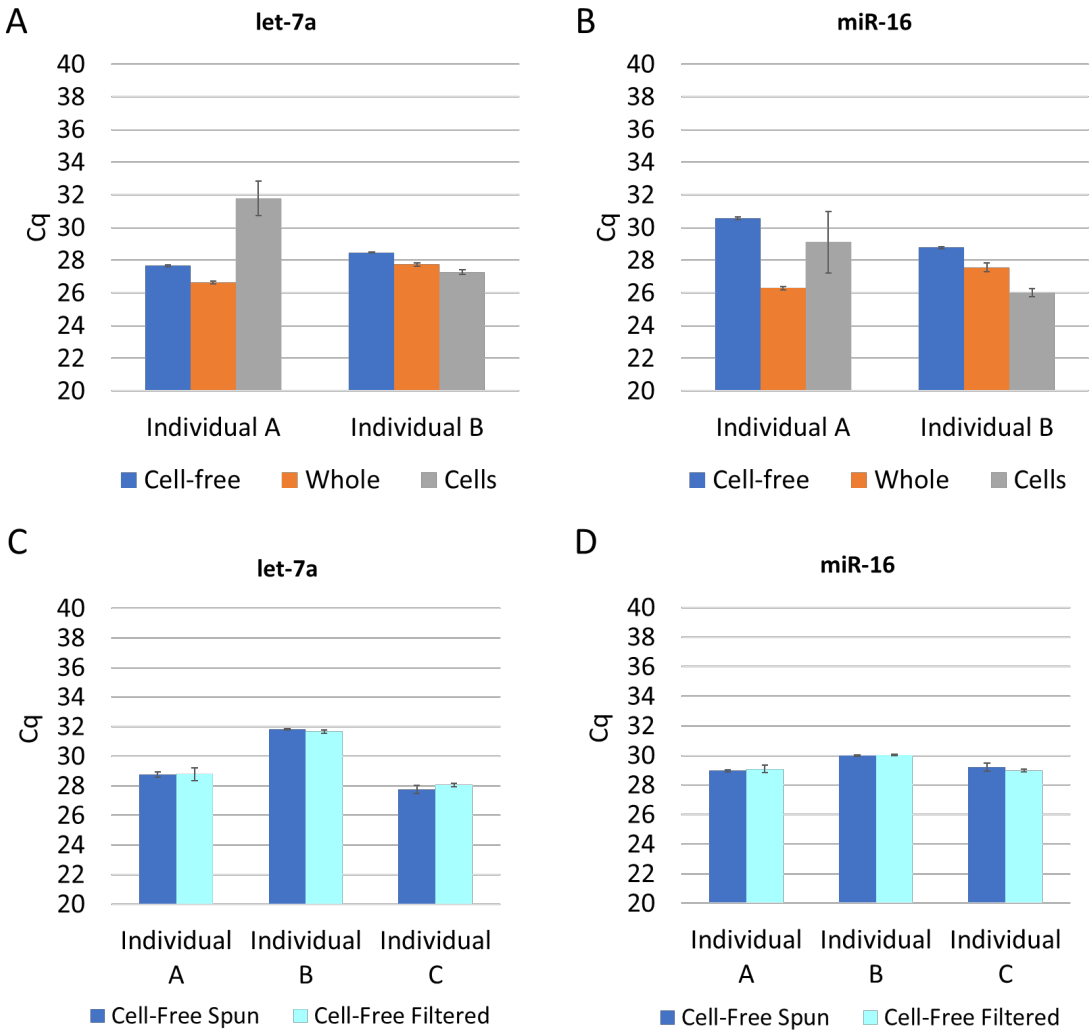

Figure 1. RT-qPCR to detect miRNAs in urine. (Panels A, B) Purifications were performed with 2x spun urine (cell-free), unspun urine (whole), and cells from 1ml of urine (cells). Mean ± standard deviation of two RT-qPCR amplifications for each of three purification replicates is shown. (A) *H. sapiens* let7-a Cq values. (B) *H. sapiens* miR-16 Cq values. (Panels C, D) Purifications were performed with cell-free urine prepared by centrifugation or by filtration. (C) *H. sapiens* let7-a Cq values. (D) *H. sapiens* miR-16 Cq values.