Promega

Product Application

Cell-free RNA extraction from plasma, urine and cerebrospinal fluid using Maxwell® RSC System

Isolate amplifiable cell-free RNA (cfRNA) from plasma, urine, and cerebrospinal fluid using the Maxwell® RSC System

Kit: Maxwell® RSC miRNA Tissue kit (Cat. #AS1460)

Analyses: RT-qPCR

Sample Types: Urine, plasma, cerebrospinal fluid (CSF)

Input: ≤300µl

Materials Required:

- Maxwell® RSC miRNA Tissue kit (Cat. #AS1460)
- 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 #TM441, available at: www.promega.com/protocols

or e-mail: techserv@promega.com

Protocol:

1. Sample preparation: Spin samples at 2,000 x *g* for 10 minutes at 4°C. Transfer cleared sample to a fresh tube and repeat the spin. NOTE: For best results do this step with fresh samples before freezing. A freeze/thaw step before centrifugation may result in cell lysis and release of gDNA into the cleared sample.
2. Add up to 300µl of cleared sample into 1.5ml microcentrifuge tubes.
3. Add 200µl of prepared Homogenization Buffer/1-Thioglycerol (at 20µl/ml) to each sample.
4. Add 200µl of Lysis Buffer and 15µl Proteinase K.
5. Vortex for 20 seconds and incubate for 10 minutes at room temperature.
6. Add entire volume to Well #1 of the Maxwell® RSC miRNA cartridge.
7. Add 10µl DNase to Well #4 and a plunger to Well #8.
8. Add 60µl of Nuclease-Free Water into each elution tubes.
9. Place cartridges and elution tubes in the Maxwell® RSC rack.
10. Run on Maxwell® RSC instrument with the RSC miRNA Tissue protocol.

Results: The above protocol was tested with 300µl and 100µl of fresh urine, fresh plasma, and previous frozen CSF per DNA extraction (n=3). Samples were amplified using miRNA and mRNA specific primers.

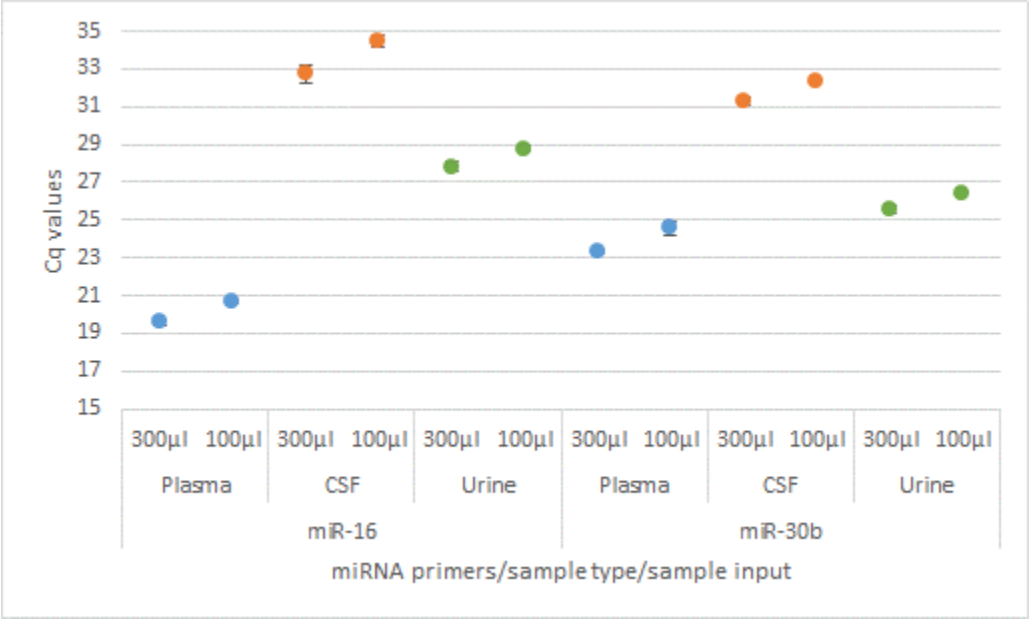

Figure 1: RT-qPCR using miRNA specific primers. Cq values from 300µl and 100µl of plasma, urine and CSF. CfDNA was isolated using the Maxwell® RSC miRNA tissue kit and amplified using RT-qPCR. Point represent the average Cq values from 3 biological replicates.

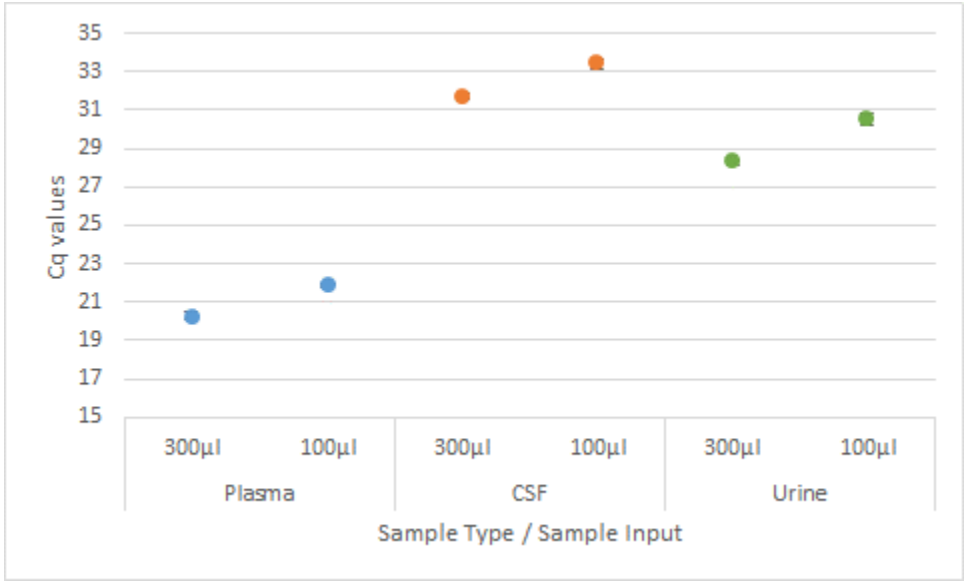

Figure 2: RT-qPCR using B2M mRNA specific primers. Cq values from 300µl and 100µl of plasma, urine and CSF. CfDNA was isolated using the Maxwell® RSC miRNA tissue kit and amplified using RT-qPCR. Point represent the average Cq values from 3 biological replicates.