Promega

Product Application

ccfDNA extraction from plasma, urine and cerebrospinal fluid using Maxwell® RSC System

Isolate amplifiable ccfDNA from plasma, urine, and cerebrospinal fluid using the Maxwell® RSC System

Kit: Maxwell® RSC ccfDNA Plasma kit (Cat. #AS1480)

Analyses: qPCR

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

Input: 1ml

Materials Required:

- Maxwell® RSC ccfDNA Plasma kit (Cat. #AS1480)
- 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 #TM454, 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 1ml of cleared sample to Well #1 of the Maxwell® RSC ccfDNA plasma cartridge.
3. Add a plunger to Well #8 and 60µl of Elution Buffer into each elution tubes.
4. Place cartridges and elution tubes in the Maxwell® RSC rack.
5. Run on Maxwell® RSC instrument with the RSC ccfDNA protocol.

Results: The above protocol was tested with 1ml of fresh urine, fresh plasma, and previous frozen CSF per DNA extraction (n=3).

Table 1: Concentrations of extracted ccfDNA determined by qPCR using 75bp amplicon primers.

	Mean (ng/µl)	STD
Plasma	0.064	0.008
Urine	0.027	0.001
CSF	1.897	0.177

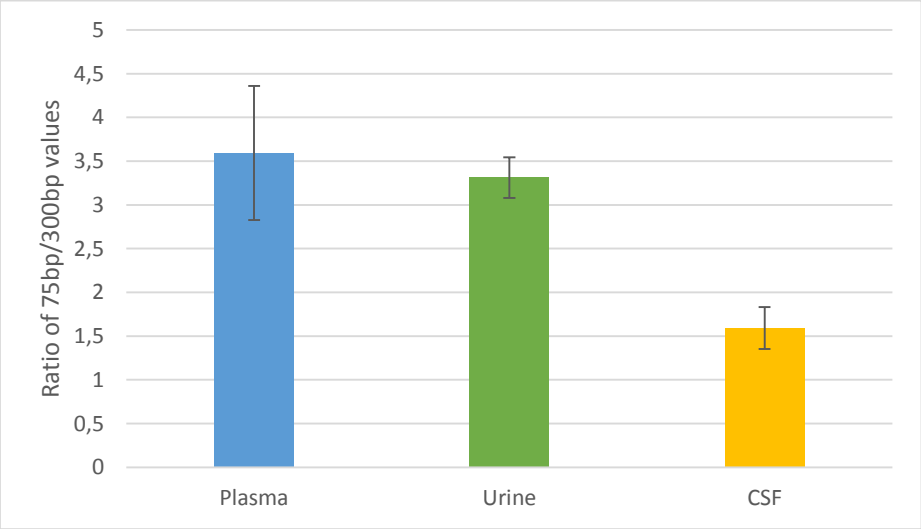

Figure 1: qPCR ratios using 75bp and 300bp amplicon primers for analysis of quality of the ccfDNA. Ratios above 1 indicates smaller DNA fragments than longer fragments. Since ccfDNA is typically small, the larger the ratio indicates more ccfDNA and less gDNA.

