Promega

Product Application

Automated purification of ccfDNA from plasma, urine and cerebrospinal fluid

Purify ccfDNA from plasma, urine, and cerebrospinal fluid using the Maxwell® RSC instrument and Maxwell® RSC ccfDNA Plasma Kit

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.

For further information, see Technical Manual TM454, available at: **www.promega.com/protocols**

or contact Technical Services at: **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

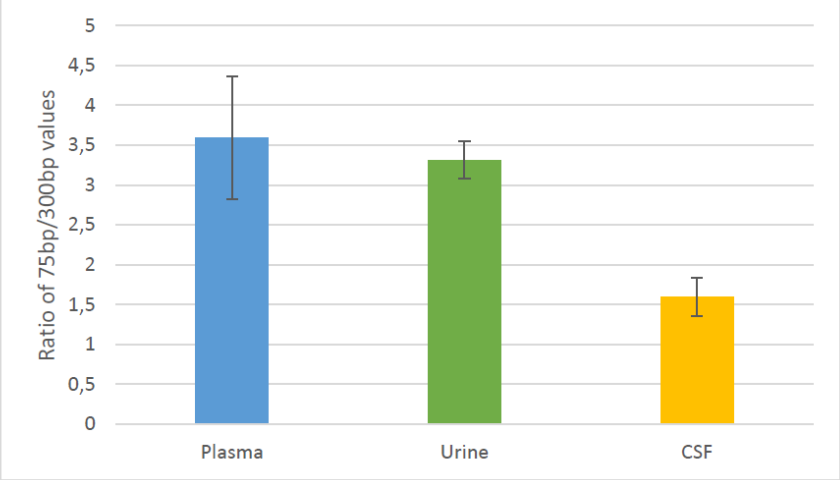

Figure 1: Analysis of quality of the ccfDNA using ProNex® DNA QC assay (Cat.# NG1002). qPCR ratios using 75bp and 300bp amplicon primers was used to determine the analysis of quality of the ccfDNA. Ratios above 1 indicates smaller DNA fragments than longer fragments. Since ccfDNA is typically small, the larger the ratio indicates more ccfDNA and less gDNA.