Promega

Product Application

Automated Purification of Viral DNA and RNA from 1ml Plasma

Purify viral DNA and RNA from 1ml of plasma 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: qPCR, RT-qPCR

Sample Type(s): Plasma

Input: 1ml

Materials Required:

- Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block (set to 37°C)

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**

Protocol:

Note: This Application Note follows the standard protocol described in the Maxwell® RSC miRNA Plasma and Serum Kit Technical Manual (TM546), increasing the sample input to 1ml of plasma.

1. Add 80µl of Proteinase K and 230µl of Lysis Buffer C to 1ml of plasma. Mix by vortexing for 5 seconds.
2. Incubate at 37°C for 15 minutes.
3. During this time, prepare the Maxwell® RSC Cartridges as described in the technical manual (TM546), using 50µl of Nuclease-Free Water for elution. **For total nucleic acid purification (including DNA virus), omit the DNase I Solution.**
4. Transfer all the lysate to well #1 of the Maxwell® RSC Cartridge.
5. Load samples onto the Maxwell® RSC Instrument and run the Maxwell® RSC miRNA Plasma and Serum method as described in the technical manual (TM546).

Results:

Serially diluted CMV- and HCV-infected samples were purified in duplicate from 1ml plasma using the Maxwell® RSC miRNA Plasma and Serum Kit on a Maxwell® RSC Instrument or using the MagNA Pure LC Total Nucleic Acid Isolation Kit – Large Volume (Roche) on a MagNA Pure LC Instrument (Roche). Relative viral recovery was assessed using qPCR for the DNA virus, CMV (Fig. 1) or 2-step RT-qPCR for the RNA virus, HCV (Fig.2). The Maxwell® RSC miRNA Plasma and Serum Kit recovered higher concentrations of virus.

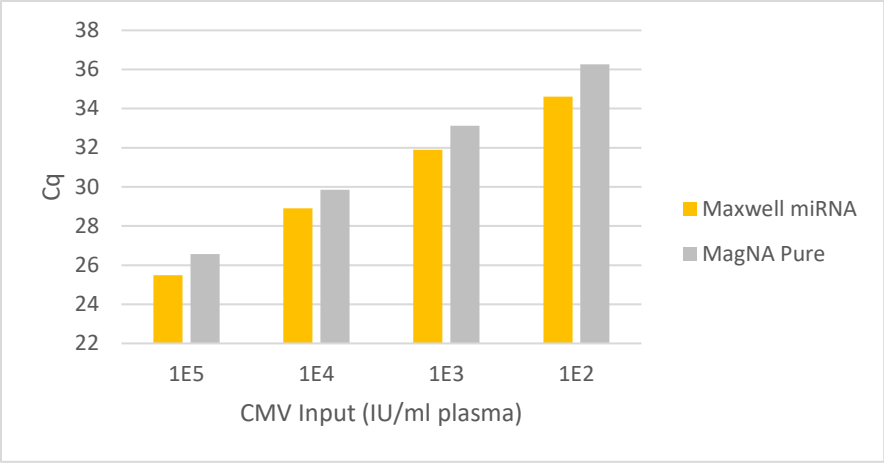

Figure 1. Relative recovery of CMV viral DNA from 1ml of human plasma using the Maxwell® RSC miRNA Plasma and Serum Kit on the Maxwell® RSC Instrument or the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche). CMV virus from a high titer patient sample (1E6 IU/ml) was serially diluted in CMV-negative human plasma and purified in duplicate with each kit. Relative performance was assessed using 5µl of eluate in qPCR with GoTaq® Probe qPCR Master Mix (Cat.# A6102) and a CMV-specific assay on the Roche LightCycler 480. Mean Cq (n=2) is shown.

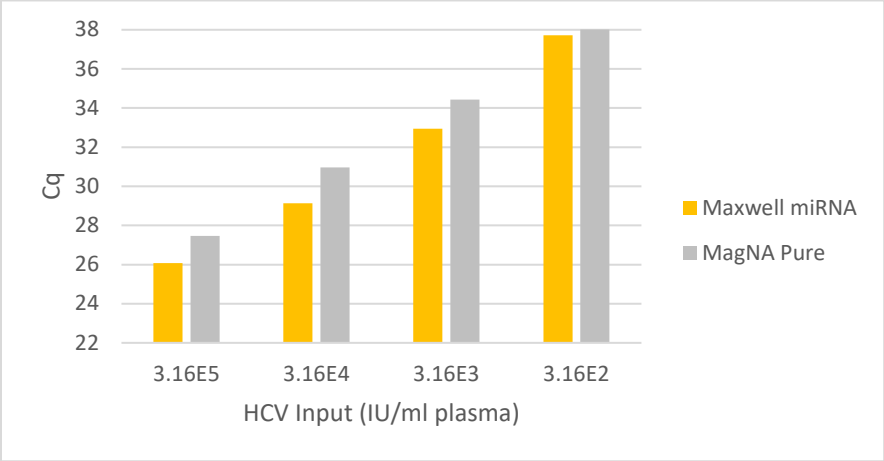

Figure 2. Relative recovery of HCV viral RNA from 1ml of human plasma using the Maxwell® RSC miRNA Plasma and Serum Kit on the Maxwell® RSC Instrument or the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche). HCV virus from a high titer patient sample (~1E5.5 IU/ml) was serially diluted in HCV-negative human plasma and purified in duplicate with each kit. Relative performance was assessed using 5µl of eluate in 2-step RT-qPCR with AMV Reverse Transcriptase (Cat.# M5108) and random hexamer primers followed by GoTaq® qPCR Master Mix (Cat.# A6002) and an HCV-specific 5' UTR assay on the Roche LightCycler 480. Mean Cq (n=2) is shown.