Promega

Product Application

Total Viral Nucleic Acid (TNA) Extraction from Whole Blood FTA Spots using the Maxwell® RSC RNA FFPE Kit

Purify viral DNA and viral RNA concurrently from whole blood spotted on FTA cards using the Maxwell® RSC RNA FFPE Kit coupled with Incubation Buffer and Proteinase K.

Kit: Maxwell® RSC RNA FFPE Kit (Cat.# AS1440)

Analyses: qPCR amplification, RT-qPCR amplification

Sample Type(s): 100µl of human blood spotted on FTA cards

Input: one to four 6mm FTA punches

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 TM436, available at: **www.promega.com/protocols**

Or contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® RSC RNA FFPE Kit (Cat.# AS1440)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Incubation Buffer (Part# D920D)
- Proteinase K (Cat.# V3021)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- Heat block
- 1.5ml tubes
- Microcentrifuge

Protocol:

Proteinase K preparation: Add 5.5ml of Incubation Buffer to the bottle of lyophilized Proteinase K, and gently swirl to dissolve. The final concentration of Proteinase K will be 18mg/ml. Dispense the stock Proteinase K solution into smaller aliquots and store at –20°C for up to 1 year. The Proteinase K can be frozen and thawed up to 5 times with no significant loss in activity. Prior to use, Proteinase K should be thawed and stored on ice

1. Add a 6mm punch to a 1.5ml microcentrifuge tube (1 to 4 punches total).
2. Add 300µl of Incubation Buffer.
3. Add 25µl of Proteinase K to sample.
4. Vortex briefly and incubate at 56°C for 30 minutes.
5. Transfer liquid and punches to a DNA IQ™ Spin Basket placed in a new microcentrifuge tube.
6. Centrifuge at max speed for 2 minutes.
7. Add the flowthrough to well #1 of a Maxwell® RSC FFPE Cartridge.
8. Place one of the supplied elution tubes into the sample rack, and add 50µl of the supplied Nuclease-Free Water for each sample.
9. Place a plunger in well #8 of each cartridge.
10. Select RNA FFPE on the Maxwell® RSC and Run.

Results:

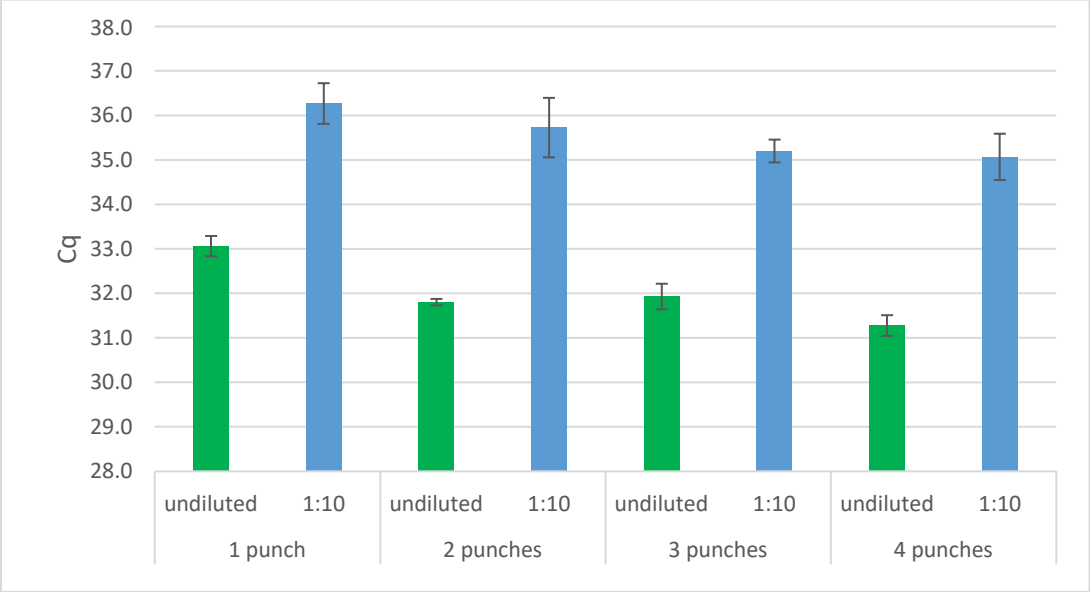

Figure 1. Average Cq of CMV Viral DNA purified from 6mm FTA punches from whole blood (spiked with NATtrol™ CMV Viral Control and MS2 RNA bacteriophage) spots using the Maxwell® RSC RNA FFPE Kit. Eluates were amplified undiluted and diluted 1:10. Cq determined by qPCR using GoTaq® Probe qPCR Master Mix (Cat.# A6101). N =3. Standard deviations are shown. All ΔCq values (difference between undiluted and 1:10 diluted sample) were ≥ 3.2, indicating no PCR inhibition was observed.

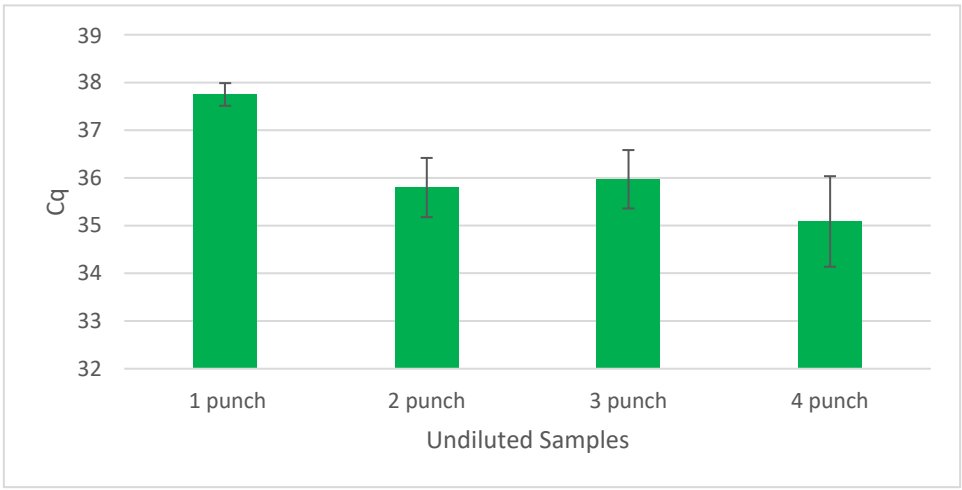

Figure 2. Average Cq of MS2 Viral RNA purified from 6mm FTA punches from whole blood (spiked with NATtrol™ CMV Viral Control and MS2 RNA bacteriophage) spots using the Maxwell® RSC RNA FFPE Kit. Eluates amplified undiluted and diluted 1:10. 1:10 dilutions amplified with ≥ 38 cycles or no Cq (data not shown). Cq determined by RT-qPCR using GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120). N =3. Standard deviations are shown.