Product Application

Purifying Total Nucleic Acid from Whole Blood Using the Maxwell® RSC Instrument

Purify qPCR-ready DNA and RNA from whole blood using an automated method. Blood should be drawn using collection tubes designed to stabilize cells, or should be processed immediately after collection, to avoid cell lysis and RNA degradation before nucleic acid can be purified.

Kit: Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses: Absorbance
qPCR and RT-qPCR

Sample Type: Whole blood, fresh

Input: 50-300µl whole blood (collected in a stabilized blood tube or processed immediately)

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 TM419, available at:
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Materials Required:

- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block

Protocol:

1. Mix blood collection tubes immediately after collection according to the manufacturer's recommendation.
2. Proceed with the protocol in the Technical Manual (TM419) to purify total nucleic acid using the Maxwell® RSC Blood DNA Kit. RNA co-purifies with this chemistry for stabilized blood samples or samples processed immediately after collection.

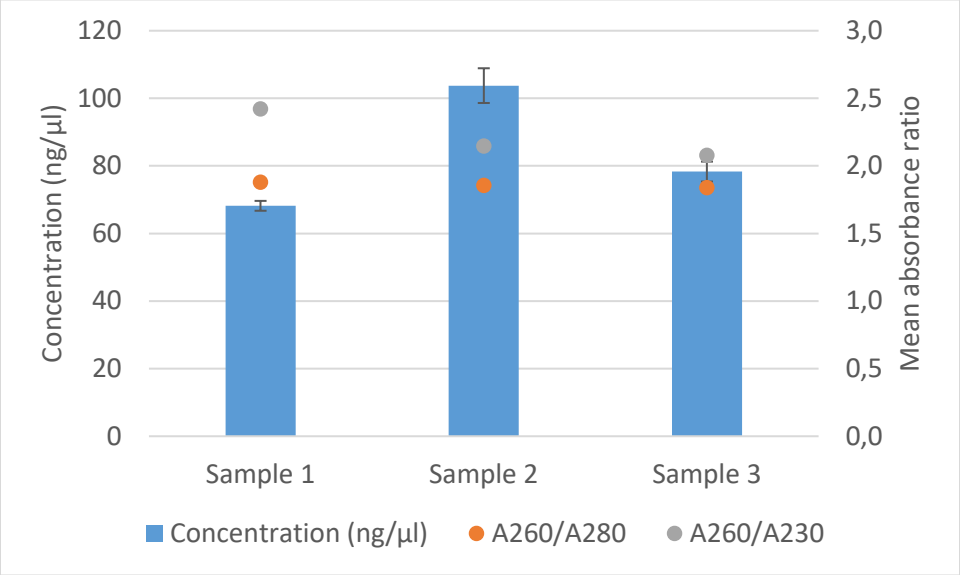

Figure 1. Concentration of total nucleic acid purified from whole blood using the Maxwell® RSC Blood DNA Kit. Blood from 3 individuals was collected in K2 EDTA tubes. The tubes were rotated for 5 minutes at room temperature and nucleic acid was purified immediately from 200µl of whole blood according to the standard protocol (n=4 each). Nucleic acid concentration was measured using absorbance on the NanoDrop® One Spectrophotometer. Mean ± standard deviation is shown for concentration; mean absorbance ratios are shown on the secondary axis.

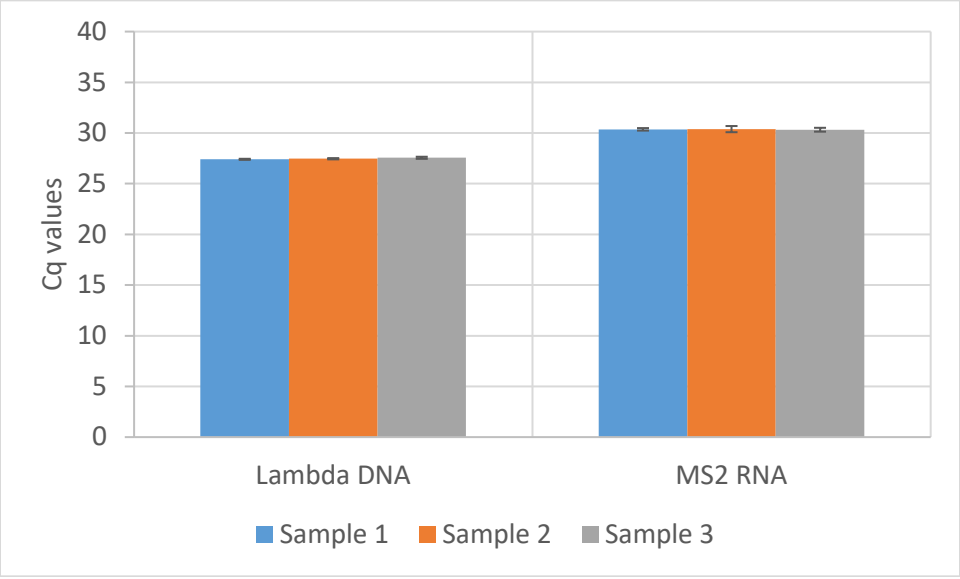

Figure 2. Purification of amplifiable DNA and RNA from 200µl whole blood purified using the Maxwell® RSC Blood DNA Kit, measured using qPCR or RT-qPCR to detect viral targets spiked into blood. Blood was collected from three individuals in K2 EDTA tubes, rotated for 5 minutes at room temperature, and spiked with Lambda bacteriophage (DNA virus) and MS2 bacteriophage (RNA virus) before purification using the standard protocol (n=4 each). Samples were then amplified using 2µl of undiluted nucleic acid in 10µl reactions with lambda-specific primers and GoTaq® Probe qPCR Master Mix (Cat.# A6101) or MS2-specific primers and GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) on a Roche LightCycler® 480 using standard cycling conditions.