Promega

Product Application

Automated DNA Purification from Blood with Low RNA Background

Purify DNA from whole blood with low RNA background using the Maxwell® RSC Blood DNA Kit and the Maxwell® RSC Instrument.

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

Analyses: Dye-based quantitation, RT-qPCR

Sample Type(s): Whole blood

Input: 300µl

Materials Required:

- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Maxwell® RSC Instrument (Cat.# AS4500)
- RNase A Solution (Cat.# A7973)
- Heat block set to 56°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 TM419, available at: **www.promega.com/protocols**

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

Protocol:

1. Mix all blood samples at room temperature for at least 5 minutes (e.g., rotate end-over-end).
2. Add 30µl of Proteinase K Solution to a 1.5ml tube.
3. Add liquid blood (up to 300µl) to each tube.
4. Add 300µl of Lysis Buffer to each tube.
5. Vortex each tube for 10 seconds.
6. Incubate each tube at 56°C for 20 minutes.
7. Prepare the cartridges as follows:
    a. Add 5µl of RNase A Solution into well #3.
    b. Place a plunger in well #8.
    c. Place an elution tube with 50µl of Elution Buffer in the deck tray.
8. Transfer each blood lysate sample to well #1 of each cartridge.
9. Run the RSC Blood DNA method on the Maxwell® RSC Instrument.

Results:

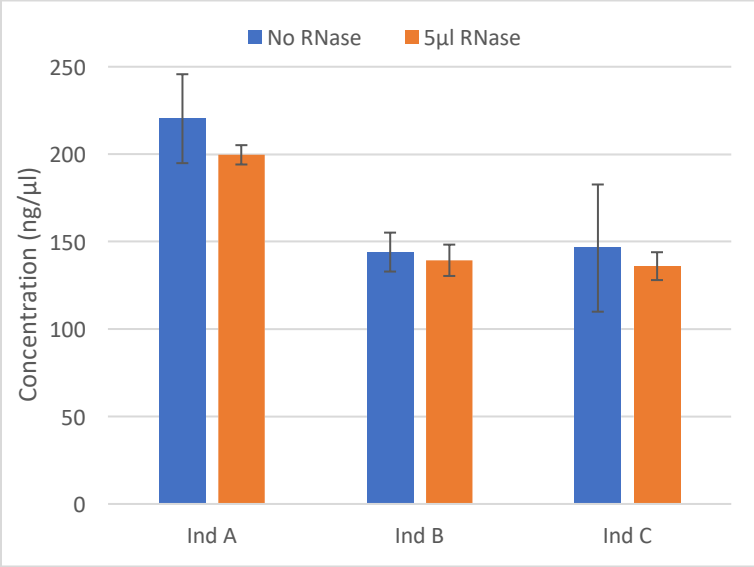
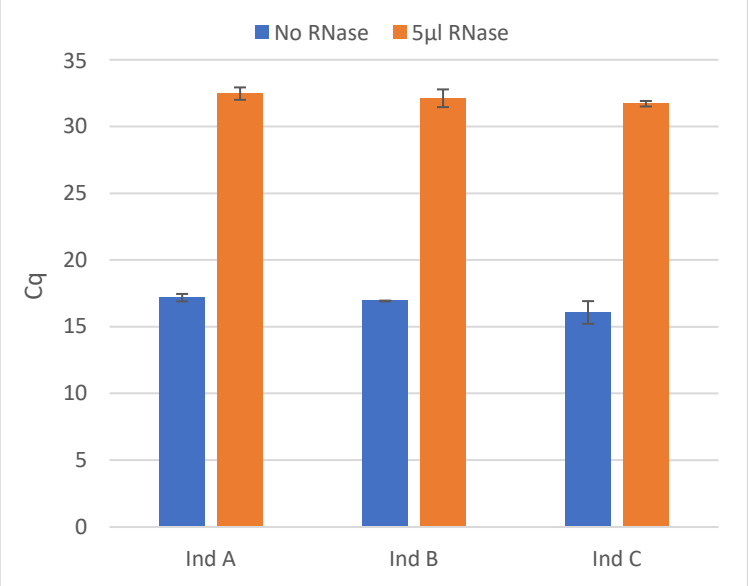


Figure 1: Concentration of DNA purified from blood. 300µl of frozen whole blood from 3 individuals was processed with and without RNase A (Cat.# A7973) using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400) according to the method described above. Blood was stored at 4°C for 3 days and then frozen at -80°C. DNA concentration was measured using the QuantiFluor® ONE dsDNA System (Cat.# E4871). Data represent the average DNA concentration of triplicate purifications with standard deviation.



Individual	Approximate Fold Difference
A	40,000
B	37,000
C	51,000

Figure 2: Detection of human RNA in DNA eluates purified from blood. 300µl of frozen whole blood from 3 individuals was processed with and without RNase A (Cat.# A7973) using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400) according to the method described above. Human RNA was detected via 1-Step RT-qPCR using the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) with B2M RNA-specific primers. Data represent the average Cq of triplicate purifications amplified in duplicate. Error bars indicate standard deviation of n=6. Approximate fold difference of RNA concentration between samples treated with and without RNase treatment is also shown. Fold difference was calculated according to the formula $2^{\Delta Cq}$ where ΔCq is the difference between the Cq of samples processed with and without RNase treatment.