



Product Application

RNA purification from buffy coat samples on the Maxwell® RSC

Purify high quality, amplifiable RNA from buffy coat samples using the Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)

Kit:	Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)
Analyses:	UV absorbance, RT-qPCR, TapeStation analysis
Sample Type(s):	Buffy coat suspension from fresh whole blood in EDTA collection tubes
Input:	Up to 100µl of buffy coat
Materials Required:	<ul style="list-style-type: none">▪ Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)▪ 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 #TM417, available at: www.promega.com/protocols

or contact Technical Service at: techserv@promega.com

Protocol:

1. Add 1-Thioglycerol to Homogenization Buffer as described in TM417.
2. Add 200µL 1-Thioglycerol/Homogenization solution to up to 100µl of buffy coat and vortex.
3. Add 200µL of Lysis Buffer and 25µL of Proteinase K.
4. Incubate at room temperature for 10 minutes.
5. Add lysate to well #1 of Maxwell® cartridge.
6. Prepare Maxwell cartridges as described in TM417 and run Maxwell® RSC simplyRNA protocol.



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Results: RNA was isolated from 100, 50, and 25 μ l buffy coat samples using the protocol above. Buffy coat was prepared by collecting fresh blood in EDTA tubes followed by centrifugation and buffy coat layer isolation.

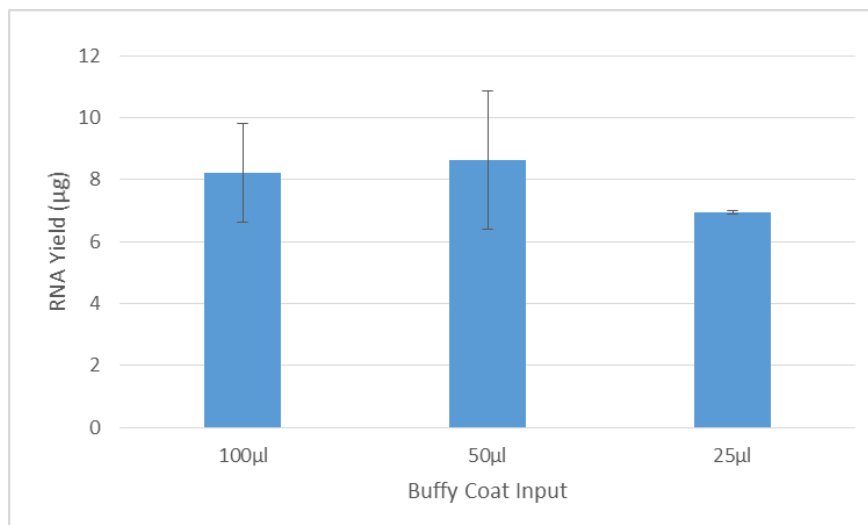


Figure 1: RNA yield determined by RT-qPCR. RNA yield was determined by RT-qPCR with RNA specific primers using a human RNA standard curve. Shown is the mean \pm STD of n=2 for each sample input.

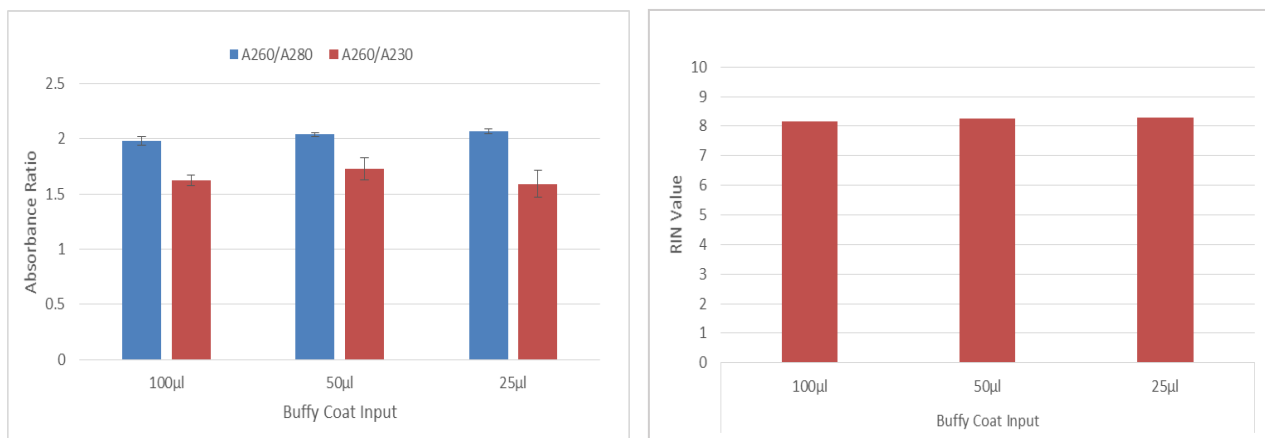


Figure 2: Absorbance ratios and RIN values of RNA eluates isolated from buffy coat samples.

Left: Absorbance ratios measured by spectrophotometry on a NanoDrop™ 1000. Shown is the mean \pm STD of n=2 for each condition. Right: RNA quality was measured on an Agilent Bioanalyzer and reported as RIN values (10 being the highest possible value).