Promega

Product Application

In collaboration with: TRAJAN

Automated RNA Purification from Blood on a Mitra® Device with the Maxwell® RSC simplyRNA Blood Kit

Purify RNA from whole blood collected on 10µl, 20µl, or 30µl VAMS® tips in a Mitra® Device using the Maxwell® RSC simplyRNA Blood Kit and the Maxwell® RSC Instrument.

Kit: Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)

Analyses: Dye-based quantitation, TapeStation, RT-qPCR

Sample Type: Blood samples collected using VAMS® tips in a Mitra® Device

Input: One 10µl, 20µl, or 30µl VAMS® tip in a Mitra® Device

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

Contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- CW Spin Baskets (Cat.# AS8101)
- CW Microfuge Tubes (Cat.# AS8201)
- VAMS® tip in a Mitra® Device (Neoteryx® by Trajan, Cat.# VM-104SNLR, VM-204SNLR, or VM-304SNLR)

Protocol:

1. Place one 10µl, 20µl, or 30µl VAMS® tip from a Mitra® Device into a CW Spin Basket inside a CW Microfuge Tube.
2. Prepare a 1-Thioglycerol/Homogenization Solution mixture by adding 20µl of 1-Thioglycerol per 1ml of Homogenization Solution. Chill on ice before use. 200µl of this mixture is required for each sample.
3. Add 200µl of 1-Thioglycerol/Homogenization Solution mixture, 200µl of Lysis Buffer, and 25µl of Proteinase K to the CW Spin Basket. Vortex for 5-10 seconds.
4. Ensure the VAMS® tip is fully submerged, then incubate at room temperature for 10 minutes.
5. Centrifuge at maximum speed for 5 minutes to collect the lysate at the bottom of the tube.
6. Transfer the full lysate to well #1 of the Maxwell® RSC simplyRNA Blood Kit cartridge.
7. Prepare the Maxwell® RSC cartridge:
    a. Place an RSC Plunger into well #8 of each cartridge.
    b. Place an elution tube with 50µl of Nuclease-Free Water into the Elution Tube position for each cartridge in the deck tray.
    c. Add 10µl of reconstituted DNase I (blue) to well #4 (yellow) of each cartridge.
8. Run the Maxwell® RSC RNA Blood Kit method on a Maxwell® RSC Instrument.

Results: RNA was successfully purified from blood collected on 10µl, 20µl, and 30µl VAMS® tips stored at room temperature for 2 hours using the method described above (Figure 1). RNA was also successfully purified from blood collected on 30µl VAMS® tips stored at room temperature for 2 hours, 24 hours, and 1 week (Figure 2).

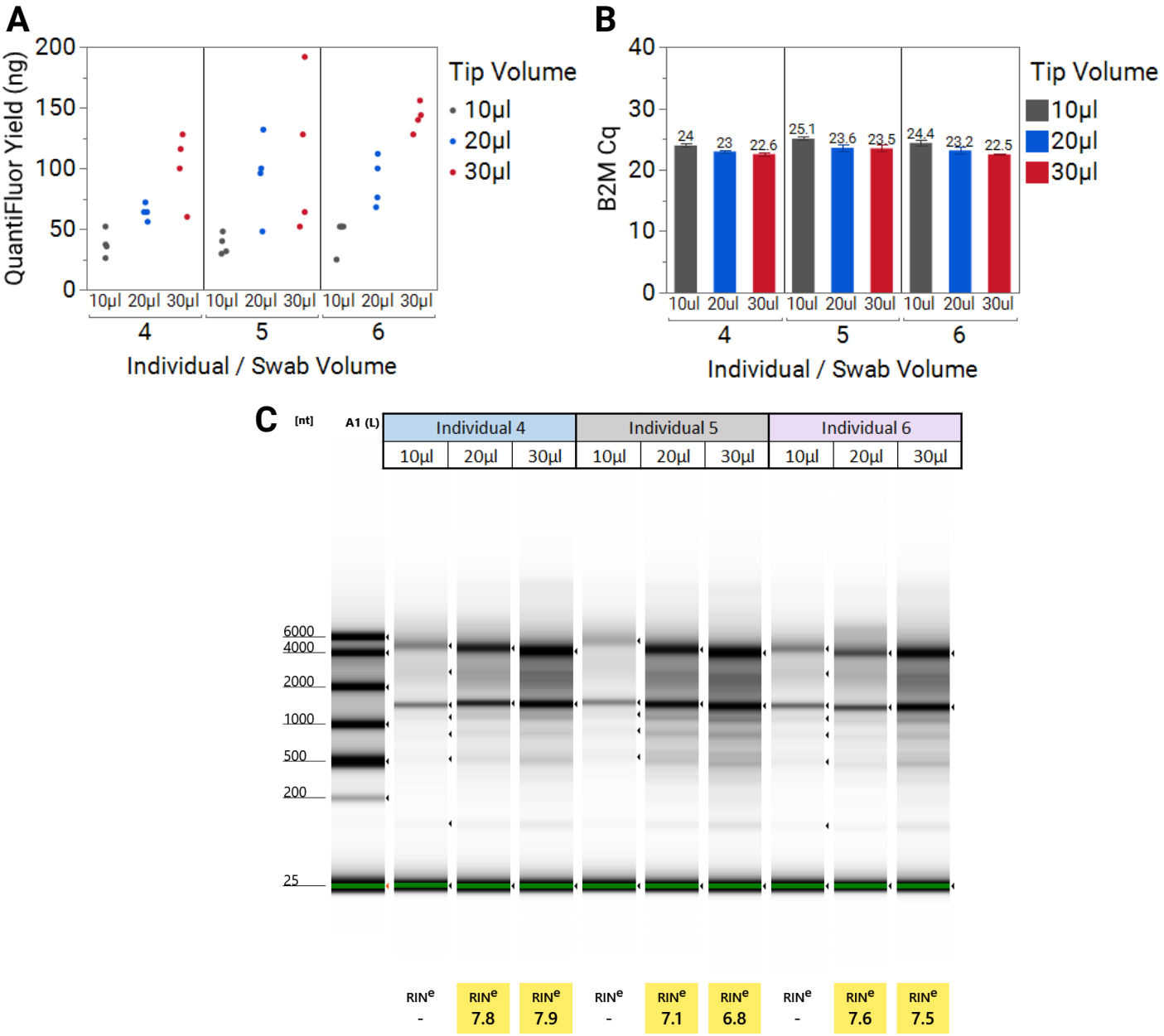

Figure 1. Assessing yield, amplifiability, and integrity of RNA eluates purified from 10µl, 20µl, or 30µl VAMS® tips stored at room temperature for 2 hours. RNA was purified from blood collected on a 10µl, 20µl, or 30µl VAMS® tip in a Mitra® Device in quadruplicate from three individuals using the method described above. After sample collection, the VAMS® tips were allowed to dry at room temperature for 2 hours before RNA purification. **A. RNA Yield.** RNA was quantified using an RNA-binding fluorescent dye (QuantiFluor® RNA System, Cat.# E3310) and yields were calculated using the recovered eluate volume. **B. RNA Amplifiability.** Eluates were amplified with the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A3120) and RNA-specific primers and probe targeting β2M mRNA (high expression gene target). Data represents the average Cq value and standard deviation for quadruplicate purifications amplified in duplicate (n=8). **C. RNA Integrity.** One purification replicate from each individual and tip volume was analyzed using the High Sensitivity RNA ScreenTape Assay on the 4200 TapeStation System. 10µl samples were below the required concentration for RIN^e score calculation.

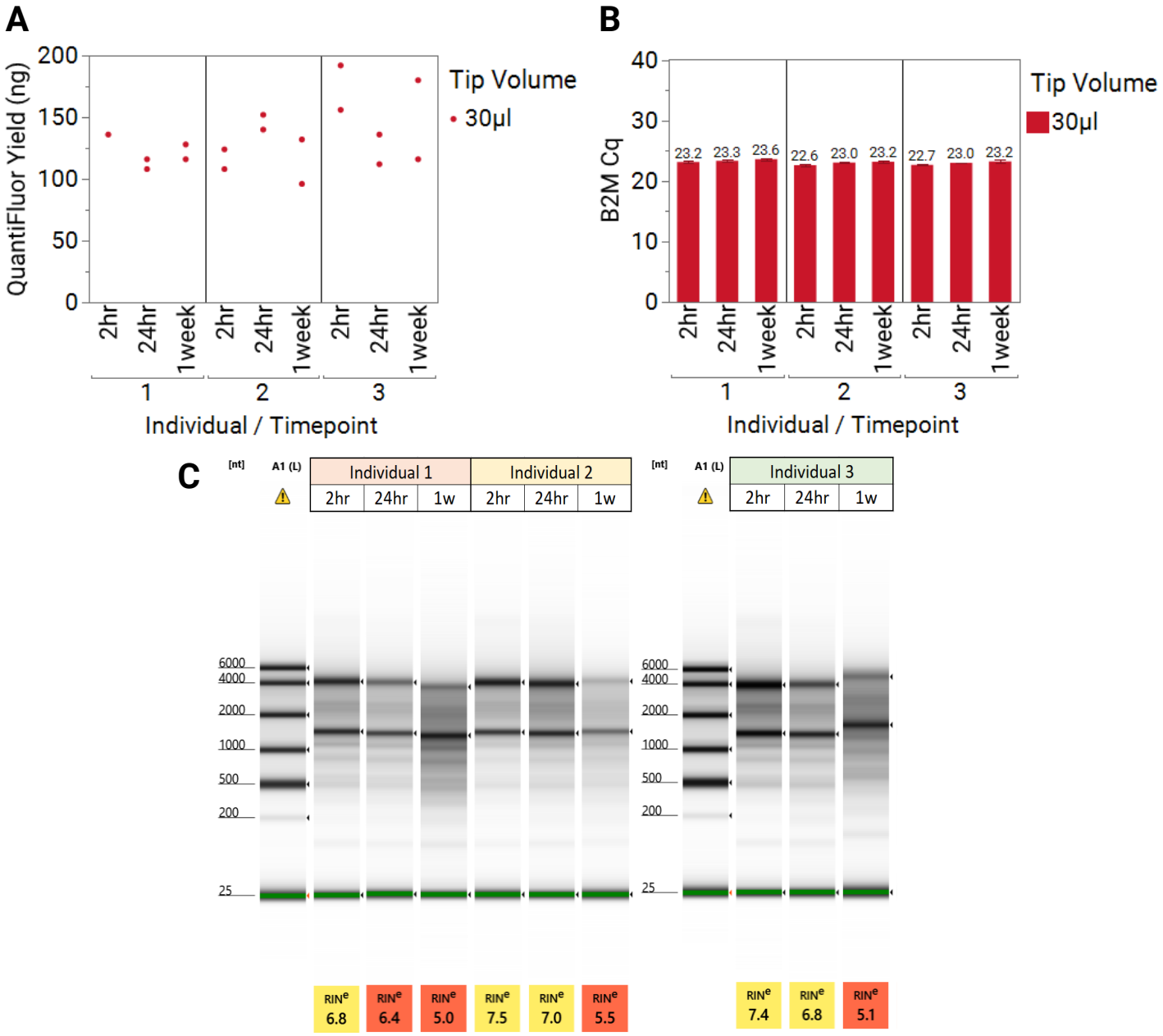

Figure 2. Assessing yield, amplifiability, and integrity of RNA eluates purified from 30µl VAMS® tips stored at room temperature for 2 hours, 24 hours, or 1 week. RNA was purified from blood collected on a 30µl VAMS® tip in a Mitra® Device in duplicate from three individuals using the method described above. After sample collection, the VAMS® tips were allowed to dry at room temperature for 2 hours, 24 hours, or 1 week before RNA purification. **A. RNA Yield.** RNA was quantified using an RNA-binding fluorescent dye (QuantiFluor® RNA System, Cat.# E3310) and yields were calculated using the recovered eluate volume. **B. RNA Amplifiability.** Eluates were amplified with the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A3120) and RNA-specific primers and probe targeting β2M mRNA (high expression gene target). Data represents the average Cq value and standard deviation for duplicate purifications amplified in duplicate (n=4). **C. RNA Integrity.** One purification replicate from each individual and timepoint was analyzed using the High Sensitivity RNA ScreenTape Assay on the 4200 TapeStation System. Warning symbols indicate that ScreenTapes were used more than 2 weeks after being first opened.