Promega

Product Application

Automated Total RNA Purification from Peripheral Blood Mononuclear Cells (PBMC)

Purify total RNA including miRNA from PBMCs using the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)

Analyses:

- Dye-based quantitation
- RT-qPCR

Sample Type(s): PBMCs

Input: ≤ 10 million cells

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)

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.

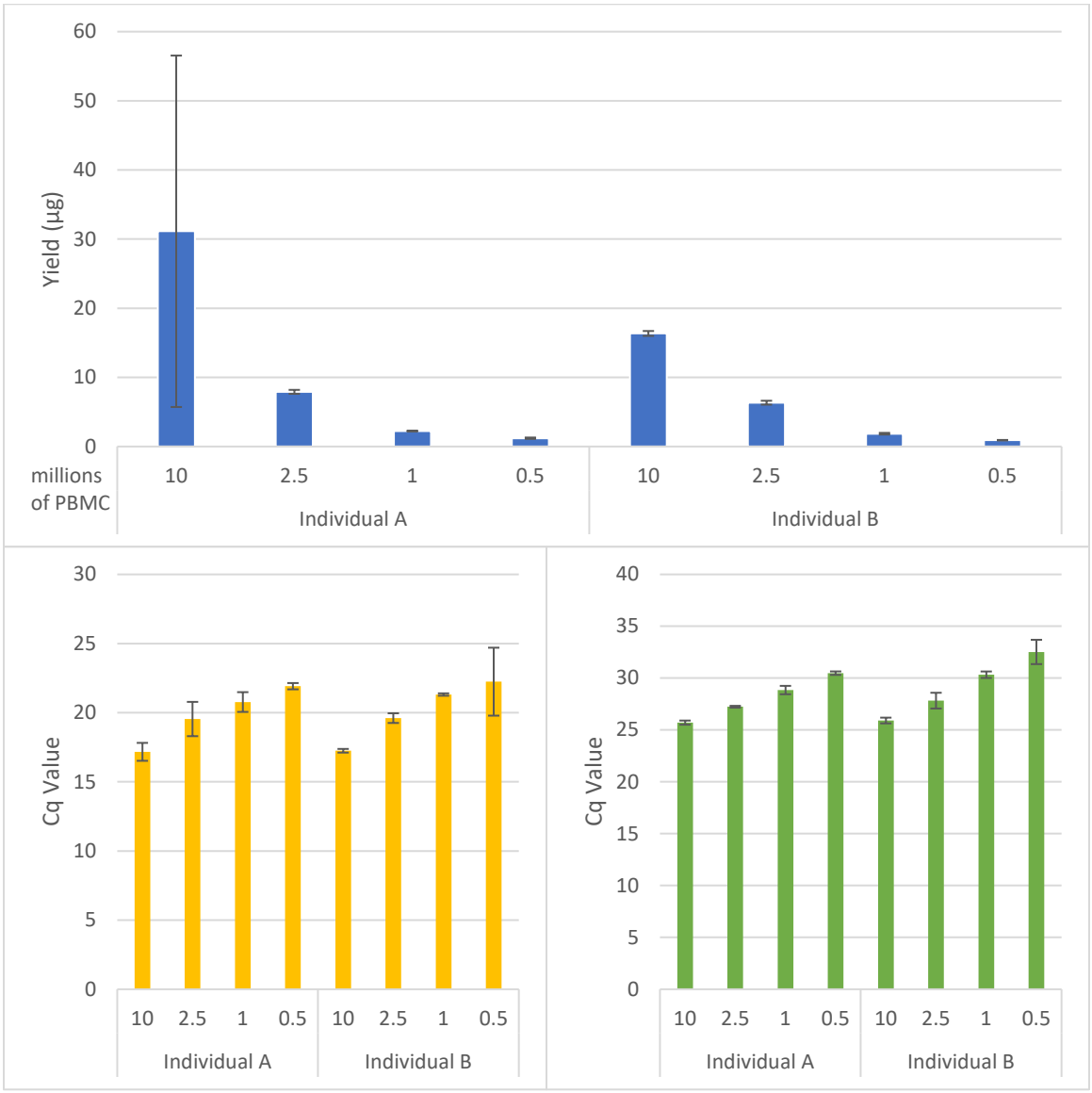
For further information, see Technical Manual TM441, available at: **www.promega.com/protocols**

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

Protocol:

1. Homogenize PBMC cell pellet in 200µl cold Homogenization Solution + 2% 1-thioglycerol.
2. Proceed with the protocol described in the Maxwell® RSC miRNA Tissue Kit Technical Manual (TM441) for the manual preprocessing steps and for preparation and loading of the Maxwell® RSC miRNA Tissue cartridges.
3. Run the miRNA Tissue protocol on the Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument.

Results:



Yield and amplifiability of RNA purified from PBMCs with the Maxwell® RSC miRNA Tissue Kit. PBMCs were isolated from the whole blood of two individuals. RNA was purified from previously frozen pellets containing the equivalent of 0.5, 1, 2.5, or 10 million PBMCs with the Maxwell® RSC miRNA Tissue Kit on the Maxwell® RSC Instrument as described in the protocol above. The concentration of the eluates was determined by dye-based quantitation with the QuantiFluor® RNA System (Cat.# E3310), and yield was determined based on the concentration and approximate elution volume recovered (*top*). Data for RNA quantitation represents the average yield for triplicate purifications ± standard deviation. To assess RNA amplifiability, samples were diluted in Nuclease-Free Water, then amplified with the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and RNA-specific primers targeting B2M mRNA (*bottom left*). Data for RNA amplifiability represent the average Cq value ± standard deviation for triplicate purifications assayed in duplicate. A TaqMan™ miRNA Assay for let-7a (ThermoFisher Assay ID 000377) was used to assess the presence of miRNAs in the eluates (*bottom right*). Data for the let-7a assay represent the average Cq value ± standard deviation for triplicate purifications with duplicate amplifications.