Promega

Product Application

Automated RNA Purification from Peripheral Blood Mononuclear Cells (PBMC)

Purify RNA from PBMCs using the Maxwell® RSC simplyRNA Cells Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC simplyRNA Cells Kit (Cat.# AS1390)

Analyses:

- Dye-based quantitation
- RT-qPCR

Sample Type(s): PBMCs

Input: ≤ 10 million cells

Materials Required:

- Maxwell® RSC simplyRNA Cells Kit (Cat.# AS1390)
- 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.

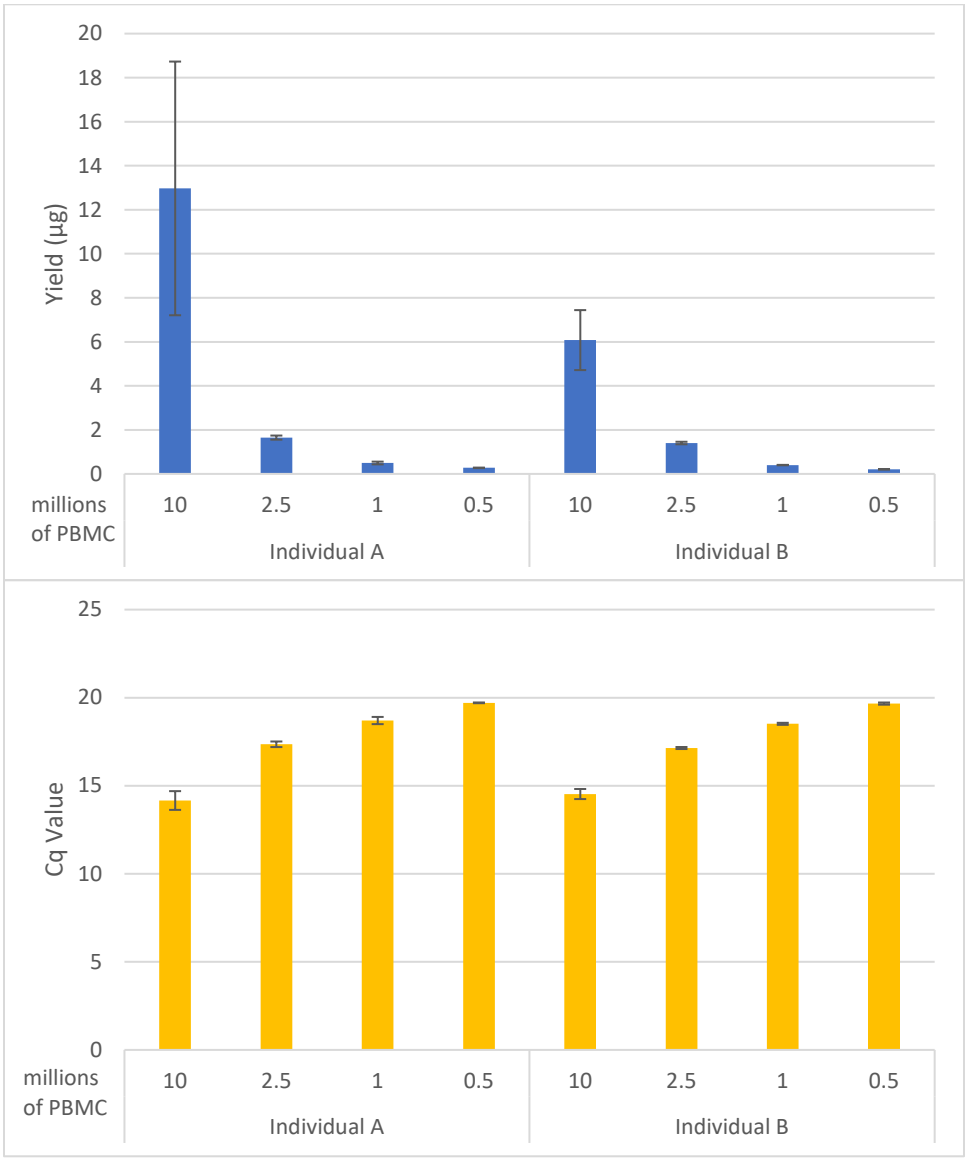
For further information, see Technical Manual TM416, available at: **www.promega.com/protocols**

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 simplyRNA Cells Kit Technical Manual (TM416) for the manual preprocessing steps and for preparation and loading of the Maxwell® RSC simplyRNA Cells cartridges.
3. Run the simplyRNA Cells protocol on the Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument.

Results:



Yield and amplifiability of RNA purified from PBMCs with the Maxwell® RSC simplyRNA Cells 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 simplyRNA Cells 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 represent the average yield for triplicate purifications ± standard deviation. To assess RNA amplifiability, samples were diluted 1:10 in Nuclease-Free Water, then amplified with the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and RNA-specific primers targeting B2M mRNA (*bottom*). Data for RNA amplifiability represent the average Cq value ± standard deviation for triplicate purifications assayed in duplicate.