

Automated DNA Purification from Peripheral Blood Mononuclear Cells (PBMC)

Purify DNA from PBMCs using the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument.

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

Analyses:

- Dye-based quantitation
- qPCR

Sample Type(s): PBMCs

Input: ≤ 5 million cells

Materials Required:

- Maxwell® RSC Blood DNA Kit (Cat. # AS1400)
- 1X PBS, pH 7.0
- 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 TM419, available at:

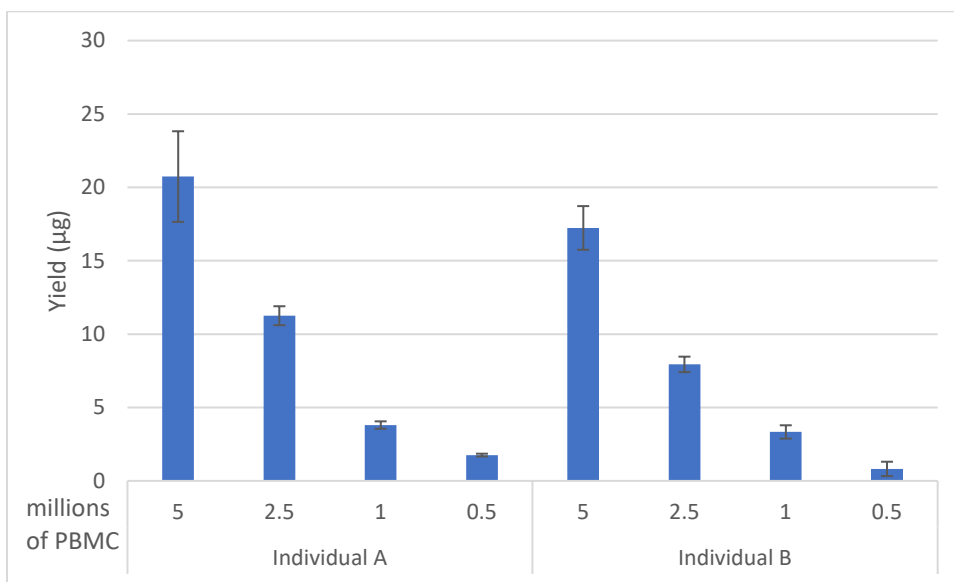
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Protocol:

1. Resuspend PBMC cell pellet in 200µl cold 1X PBS, pH 7.0 by vortexing.
2. Treat the cells suspended in PBS as a blood sample, and proceed with the protocol described in the Maxwell® RSC Blood DNA Kit Technical Manual (TM419) for the manual preprocessing steps and for preparation and loading of the Maxwell® RSC Blood DNA cartridges.
3. Run the Blood DNA protocol on the Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument.

Results:



Yield of DNA purified from PBMCs with the Maxwell® RSC Blood DNA Kit. PBMCs were isolated from the whole blood of two individuals. DNA was purified from previously frozen pellets containing the equivalent of 0.5, 1, 2.5, or 5 million PBMCs with the Maxwell® RSC Blood DNA Kit (Cat.# AS1400) as described in the protocol above. The concentration of the eluates was determined by amplification with the ProNex® DNA QC Assay (Cat.# NG1002 or Cat.# NG1004) , and yield was determined based on the concentration and approximate elution volume recovered. Data for the ProNex® DNA QC Assay represent the average yield value \pm standard deviation for triplicate purifications with duplicate amplifications. The ProNex® DNA QC Assay also showed that the DNA was intact with undetectable amounts of degradation and that there is no detectable amplification inhibition in the eluates (data not shown).