Technical Information

RNeasy® Plus Kit

Analysis of yield, quality and DNA depletion of total RNA purified from peripheral mononuclear cells (PBMCs) using the RNeasy Plus Kit

Introduction

Drug discovery and biomedical research advance with the aid of high-quality human biological specimens. One of the most versatile specimen types is peripheral blood mononuclear cells (PBMC). PBMC are characterized by a round nucleus and consist mainly of lymphocytes (T cells, B cells, and NK cells), macrophages and dendritic cells. The aim of this study was to analyze the yield and overall quality of total RNA extracted from PBMC using the RNeasy Plus Mini Kit. This staple of the research lab integrates fast and convenient RNA purification with effective elimination of genomic DNA contamination to provide higher RNA yield and quality compared to alternative suppliers.

Materials and Methods

Blood was drawn into lithium-heparin collection tubes and PBMC were isolated following the QIAGEN Supplementary Protocol "Isolation of Peripheral Blood Mononuclear Cells (PBMC) and Purification of Total RNA from PBMC". This protocol isolates PBMC from whole blood by density centrifugation using Ficoll-Paque as a density-gradient medium. In two independent experiments, total RNA was extracted from 4 aliquots of 5 x 10^6 isolated PBMC each using the standard protocol of the RNeasy Plus Mini Kit and the standard protocols of two alternative suppliers (supplier A and B). RNA quality was analyzed on a 0.8% agarose gel. Concentration and A_{260}/A_{280} ratio were determined on the QIAxpert®, an innovative high-speed microfluidic UV/VIS spectrophotometer for accelerated DNA, RNA, and protein quantification and quality control. RNA integrity was analyzed using the Agilent® RNA 6000 Nano Kit on the Agilent 2100 Bioanalyzer. Amount of gDNA contamination in the purified RNA was determined by real-time PCR amplification of the HOXD9 gene using the QuantiNova® SYBR Green PCR Kit.

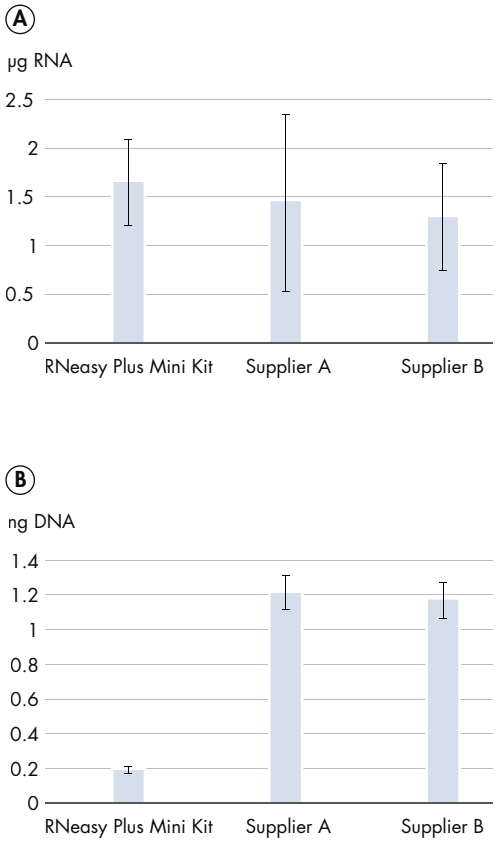

Figure 1. Consistently high yields and efficient gDNA removal with the RNeasy Plus Mini Kit. A Mean yield of RNA purified from 5 x 10^6 PBMC using the RNeasy Plus Mini Kit and solutions from two alternative suppliers. Yields were determined using the QIAxpert and exhibited decreased variability for the RNeasy Plus Mini Kit compared to Suppliers A and B. **B** A real -time PCR assay for HOXD9 was performed on the extracted RNA to determine the amount of residual gDNA. Contamination was significantly lower in the RNA purified with the RNeasy Plus Mini Kit.

Results

Consistent RNA yields and effective gDNA removal

The RNeasy Plus Mini Kit and the tested solutions from alternative suppliers use gDNA removing columns for rapid and effective elimination of gDNA contamination. Smart analysis protocols installed on the QIAxpert enable specific quantification of analytes of interest and determined an average RNA yield from 5 x 10^6 PBMC of 1.65 µg for the RNeasy Plus Mini Kit, 1.44 µg for supplier A, and 1.29 µg for Supplier B (Figure 1A). The RNeasy Plus Mini Kit provided the most consistent RNA yields (lower variance). Real-time PCR amplification of the HOXD9 gene revealed significantly lower gDNA contamination in RNA purified with the RNeasy Plus Mini Kit compared to solutions from Suppliers A and B (Figure 1B).

Compared to the tested solutions from alternative suppliers, the RNeasy Plus Mini Kit provides the most reliable RNA purification from this demanding sample type. Isolates from PBMC had higher and more consistent yields of total RNA and elimination of gDNA contamination (Figure 1B) was approximately10-fold better.

Reproducible purification of high-quality RNA

RNA integrity and quality results are shown in Figure 2. Both agarose gel analysis and scrutiny with the Agilent Bioanalyzer show high-quality RNA in all eluates. Electropherograms and gel images exhibit sharp peaks and bands for 18S and 28S ribosomal RNA (Figure 2). The RNA integrity number (RIN) values for samples prepared with the RNeasy Plus Mini Kit averaged 9.6, slightly higher compared to values obtained with the solutions from Supplier A and B (Table 1). However, the standard deviation of samples prepared with the RNeasy Plus Mini Kit was substantially lower than the alternative solutions, indicating that RNA integrity was consistently higher in these samples compared to the more variable results of the other two kits.

Table 1. Quality assessment of total RNA purified from 5x10^6 PBMC using the indicated kits

Quality criteria (average)	RNeasy Plus Mini Kit	Supplier A	Supplier B
Concentration (ng/ml)	33.18	28.88	25.75
Standard deviation	8.75	18.28	10.95
A_{260}/A_{280}	2.1	2.01	2.17
Standard deviation	0.04	0.09	0.12
RNA Integrity Number (RIN)	9.6	9.3	9.2
Standard deviation	0.16	0.43	0.66

Conclusion

Isolation of PBMC is a laborious and challenging lab procedure, making it imperative to maximize insights obtained from this specimen type. The RNeasy Plus Mini Kit provides easy and efficient purification of total RNA from PBMC with:

- Consistently high yields
- Reproducibly high quality
- Effective gDNA removal

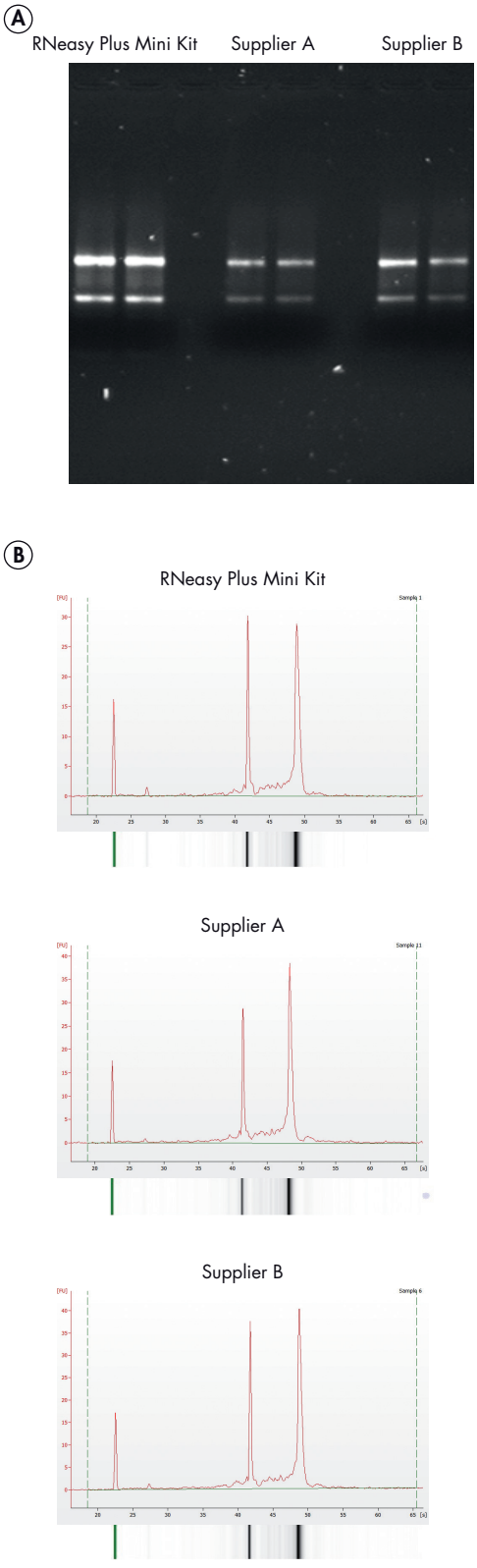

Figure 2 . Clear bands and sharp peaks are evidence of the high-quality RNA isolated from PBMC. A Gel electrophoresis and **B** analysis of eluates on the Agilent Bioanalyzer (1 µl of each eluate) reveal high-quality RNA from all three purification solutions.

Ordering Information

Product	Contents	Cat. no.
RNeasy Plus Mini Kit (50)	For 50 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-free water and buffers	74134
RNeasy Plus Mini Kit (250)	For 250 minipreps: RNeasy Mini Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, RNase-free water and buffers	74136
Relative Products		
QuantiNova SYBR Green PCR Kit (100)*	For 100 x 20 µl reactions: 1 ml 2x QuantiNova SYBR Green PCR Master Mix, 500 µl QuantiNova Yellow Template Dilution Buffer, 250 µl QN ROX Reference Dye, 1.9 ml water	208052
QIAxpert Instrument	QIAxpert instrument with 1 year warranty coverage including parts, labor and shipping; repair by sending to a regional repair center	9002340

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Maximize insights from your PBMC samples!