Application Note

QIAxpert® PAXgene® RNA Spectral Content Profiling App

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Introduction

A new analysis protocol (App) for precise quantification of human whole blood RNA is available for the QIAxpert spectrophotometer. The QIAxpert PAXgene RNA App is dedicated for quantification of RNA purified with PAXgene Blood RNA kits using manual purification or either QIAsymphony® or QIAcube® technologies.

The proprietary spectral content profiling is used to separate the profile of the RNA purified using the PAXgene Blood RNA Kits (PreAnalytiX® GmbH) from the measured UV/VIS spectrum, thereby distinguishing it from interfering chemicals and other potential contaminants. The specific RNA profile is then used to determine the RNA concentration (ng/µl) based on the absorbance at 260 nm using a concentration factor of 40. Due to spectral content profiling of the QIAxpert the purified RNA sample can be quantified directly without diluting in 10 mM Tris·Cl, pH 7.5.

In this Application Note, we describe how the App presents data and show data on performance of the QIAxpert PAXgene RNA App.

Figure 1. The QIAxpert PAXgene RNA App icon.

Materials and Methods

The PAXgene RNA App is identified by the icon shown in Figure 1. Compatible kits are listed in Table 1. ▷

Table 1. Kits compatible with the QIAxpert PAXgene RNA App*

Product	Contents	Cat. no.
PAXgene Blood RNA Kit (IVD)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes	762174/ 762164
PAXgene Blood RNA MDx Kit	For 4 x 96 RNA preps on the BioRobot® MDx workstation: 4 PAXgene 96 RNA Plates, 4 PAXgene 96 Filter Plates, Buffers (wash buffers are labeled with bar codes), Proteinase K, RNase-Free DNase Set, Plasticware, Collection Vessels. To be used with PAXgene Blood RNA Tubes	762431
PAXgene 96 Blood RNA Kit	4 PAXgene 96 RNA Plates, 4 PAXgene 96 Filter Plates, Buffers, Proteinase K, RNase-free DNase Sets, AirPore Tape Sheets, Collection Vessels. To be used in conjunction with PAXgene Blood RNA Tubes.	762331
PAXgene Blood miRNA Kit	For 50 RNA preps: PAXgene RNA MinElute® Spin Columns, PAXgene Shredder Spin Columns, Processing Tubes, Microcentrifuge Tubes, Carrier RNA, RNase-Free DNase, and RNase-Free Buffers; to be used in conjunction with PAXgene Tissue Containers	766134
QIAsymphony PAXgene Blood RNA Kit	For 96 preps: 2 Reagent Cartridges, Enzyme Racks, accessories, and RNase-free buffers	762635

* The applications with these kits are intended for molecular biology applications. They are not intended for the diagnosis, prevention, or treatment of a disease.

Profile description

The spectral content profiling algorithm in this App differentiates RNA, DNA and other impurities, as well as sample background, by analyzing the full measured UV/VIS spectrum (white curve) and provides distinct profiles as a result (Figure 2):

- RNA – shown as the blue curve – is the molecule of interest. This profile is specific for RNA.
- Impurities (orange curve) – non-RNA molecules that also absorb in the UV region (e.g., DNA, as well as protein residue, thiocyanate salts, EDTA, citrate and azide).
- Background (grey curve) – profile combining intrinsic sample turbidity, scattering from bead contamination or other particles, as well as hemoglobin/heme residue (if present, an absorbance at approximately 410 nm is visible). This background spectrum is subtracted from the measurement prior to profiling.
- Residue (yellow curve) – unidentified part of the measured spectrum, also shown as a percentage value of the measured spectrum (white curve).

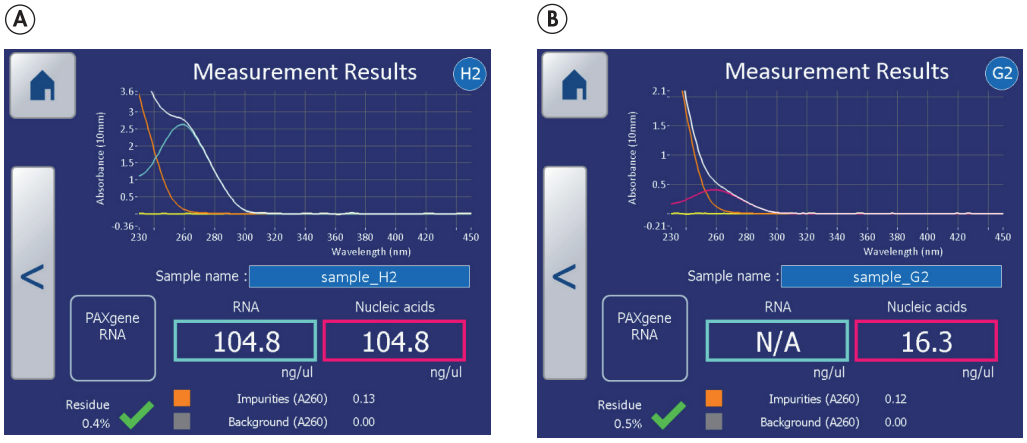

Figure 2. Example result screen for spectral content analysis. RNA was purified from human whole blood using the QIAsymphony PAXgene Blood RNA MDx Kit and the QIAxpert PAXgene RNA App was used for measurement. **A** An RNA-profiled PAXgene RNA sample with an RNA concentration of 104.8 ng/µl and a low residue value of 0.4%. **B** A PAXgene RNA sample, where RNA profiling is unavailable, due to a low nucleic acid concentration of 16.3 ng/µl, but still showing a low residue value of 0.5%.

Spectral profiling of RNA is unavailable in specific circumstances for samples with:

- A_{260} below 0.5 OD (10 mm) after background correction; i.e., if the nucleic acid concentration of the sample is below 20 ng/µl (Figure 2B)
- Residue above 2.5% due to an unknown component (a red cross instead of a green checkmark is shown next to the residue value)

In these circumstances, a total nucleic acids spectrum (pink curve) is displayed instead of the RNA profile (Figure 2B). The concentration is calculated using the A_{260} value of this profile multiplied by the concentration factor of RNA (i.e., 40).

Blanks

For spectral content profiling analysis, sample elution buffer as a blank is not required and will adversely affect the result. Required corrections for spectral content profiling are performed via an automatic blanking by the system. Alternatively, pure water (ddH_2O) can be used as the blank. For recommendations on blanks, see the *QIAxpert User Manual* (available at **www.qiagen.com/qiaxpert**).

Results

Performance data

The performance of the PAXgene RNA App was determined regarding linear regression: measured as target concentration vs. measured concentration, and precision/reproducibility: measured as standard deviation (STD [ng/µl]) and coefficient of variation (CV [%]) of multifold measurement of one sample. ▷

Linearity and precision/reproducibility characteristics of the PAXgene RNA App were determined by measuring a series of dilution of human whole blood RNA (purified using either the QIAsymphony instrument and the QIAsymphony PAXgene Blood RNA Kit, 762635 or the QIAcube instrument and the PAXgene Blood RNA Kit, 762174). Concentrations of human whole blood RNA ranging from 75 ng/µl to 0 ng/µl were prepared in elution buffer BR5. Each concentration was measured 5 times on the same QIAxpert instrument using the PAXgene RNA App with the automatic blanking option.

Linear regression

Results showed a high correlation to linear regression with a calculated regression coefficient of $R^2 = 0.998$ for the QIAsymphony-based (Figure 3A) and a regression coefficient of $R^2 = 0.999$ for the QIAcube-based (Figure 3B) PAXgene Blood RNA purification systems.

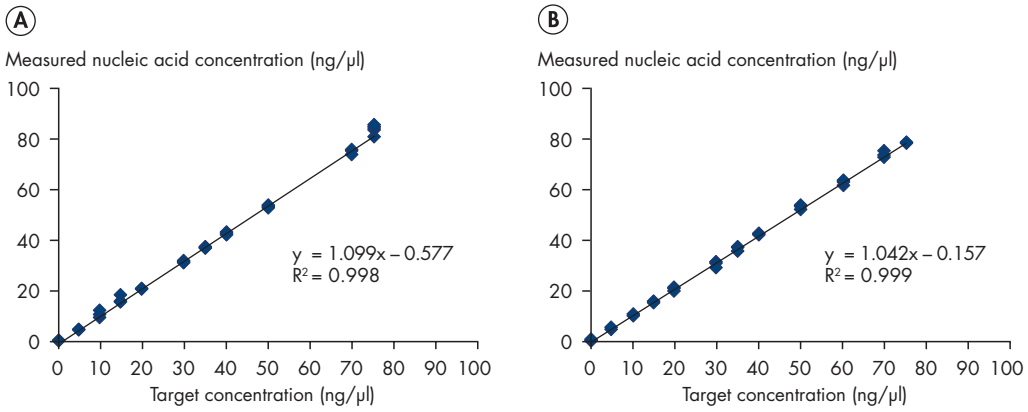

Figure 3. Linear regression of dilutions of purified human whole blood RNA. Each concentration was measured 5 times on the same QIAxpert instrument using the PAXgene RNA App with the automatic blanking option. **A** Purified with QIAsymphony using the QIAsymphony PAXgene Blood RNA Kit. **B** Purified with QIAcube using the PAXgene Blood RNA Kit.

Precision/reproducibility

Precision and reproducibility of the QIAxpert PAXgene RNA App was evaluated for the 5 repeated measures with concentrations from 75 ng/µl down to 5 ng/µl. STD (ng/µl) and CV (%) were calculated for each concentration as a measure of precision.

Variability within the 5 repeated measurements for each dilution was low, with STD (ng/µl) consistently below 1 ng/µl and CV (%) below 1.5% for many of concentrations tested (Figure 4). Only for the lower concentrations (≤15 ng/µl or ≤30 ng/µl, respectively) did CV (%) values elevate above 1.5% to 9% for RNA purified by the QIAsymphony PAXgene Blood RNA Kit and to 4.5% for RNA purified by the QIAcube/PAXgene Blood RNA Kit.

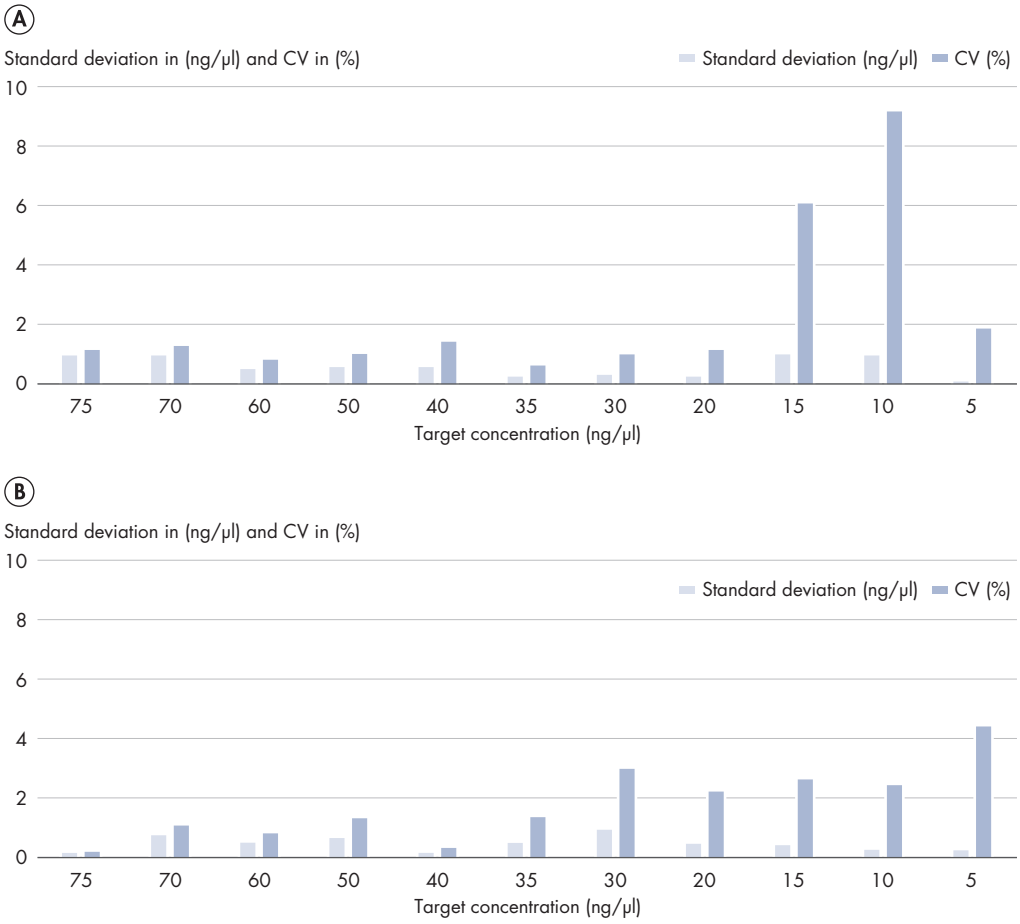

Figure 4. Precision/reproducibility of multifold measurements of dilution series of purified human whole blood RNA. The STD (ng/µl) and CV (%) was determined for each 5-fold measured concentration. The measurements were recorded on the same QIAxpert instrument using the PAXgene RNA App with the automatic blanking option. **A** Purified with QIAsymphony using the QIAsymphony PAXgene Blood RNA Kit. **B** Purified with QIAcube using the PAXgene Blood RNA Kit.

Conclusion

The QIAxpert PAXgene RNA App shows excellent performance in terms of linearity and precision/reproducibility with a limit of detection of 1.2 ng/µl (0.03 OD_{600} nm).

The App enabled precise quantification of human whole blood RNA purified by either the bead-based QIAsymphony PAXgene Blood RNA system or the silica-based QIAcube PAXgene Blood RNA system. This App is also suitable for the quantification of human whole blood RNA isolated with PAXgene Blood RNA systems (see Table 1 for compatible kits).

Overall, the QIAxpert performs nucleic acid quantification and quality control in a fast and easy manner, analyzing up to 16 samples within 2 minutes. Furthermore the use of microfluidic QIAxpert slides provides qualitative measurements by avoiding cross-contamination, as well as evaporation or meniscus effects.

Download information

Product	Contents	Link
QIAxpert PAXgene RNA App	For quantification of RNA purified with PAXgene Blood RNA kits using manual purification, QIAsymphony or QIAcube technology: the concentration (ng/µl) is determined based on the absorbance at 260 nm using a concentration factor of 40	**www.qiagen.com/qiaxpert**

The applications presented here are for molecular biology applications. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. It is the user's responsibility to validate the performance of the protocol with QIAxpert PAXgene RNA App for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. The performance characteristics of this product have not been fully established.

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