Application Note

QIAcube® RNA isolation from stool samples using the RNeasy® PowerMicrobiome® Kit

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This application note demonstrates that RNA is extracted efficiently from stool samples using the RNeasy PowerMicrobiome Kit and the QIAcube system. Furthermore, the RNA isolated with the RNeasy PowerMicrobiome Kit and the QIAcube system is compatible with downstream applications.

Introduction

Many microbes are considered 'biological dark matter' either because they have yet to be discovered or are not easily culturable, making characterization difficult. Technical developments in the field of metagenomics have enabled researchers to more deeply assess the composition of microbiomes and the species and genes that are present in the microbial community. Presently, comprehensive microbiome studies also include metatranscriptomics. In this approach, the total RNA from the microbial community is analyzed to quantify the transcriptionally active and inactive genes.

Modern sequencing techniques have opened the door to the analysis of complex microbial communities referred to as the microbiome. With increased understanding of the microbial world, it has become evident that the composition of these communities plays a significant role in health and the environment. For example, the microbiome is not only associated with infectious diseases but also with various conditions such as diabetes, obesity and even depression.

However, analyzing microbiomes still has numerous challenges. Efficient and uniform lysis of microbes is necessary to ensure that microbial community representation is as accurate as possible. Additionally, effective inhibitor removal is critical since many sample types, such as stool and soil, contain substances that inhibit enzyme activity and thereby impact performance of applications like PCR. Because the inhibitor removal and purification procedures are often time consuming, these can now be automated using the QIAcube. Automation not only saves time, but also ensures standardization.

Materials and methods

Experiment 1: Isolation of RNA from human stool samples using the RNeasy PowerMicrobiome Kit, Inhibitor Removal Technology® (IRT) protocol, with on-column DNase digest on the QIAcube.

RNA was isolated from human stool samples using either the manual protocol or the QIAcube IRT protocol with the RNeasy PowerMicrobiome Kit. For both protocols 200 mg from the same human stool sample were placed into PowerBead Tubes. Cells were lysed according to the kit manual by adding 650 µl Solution PM1 with β-mercaptoethanol and 100 µl phenol/chloroform/isoamylalcohol, followed by vortexing for 10 minutes at maximum speed using a 24-sample vortex adapter. To allow for a reliable comparison between manual and automated sample processing, supernatants were pooled after mechanical lysis. This minimizes the impact of sample-to-sample variation when working with heterogeneous sample material such as stool. From this pool of supernatants, 450 µl per sample was then processed with the manual and QIAcube protocols according to the instructions in the *RNeasy PowerMicrobiome Kit Handbook*. Briefly, for the manual protocol, after mechanical lysis IRT was used to remove contaminants from the stool sample, followed by binding of nucleic acids to a silica-based spin column, on-column DNase digest and washing and elution in 100 µl of RNase-free water. For the QIAcube protocol, after mechanical lysis, 450 µl per sample was transferred into position 2 of the rotor adapter which was then placed into the centrifuge of the QIAcube. All subsequent steps including the removal of inhibitory substances and on-column DNase digestion were performed on the QIAcube. For each protocol, 6 samples were processed. After isolation, RNA yield was determined by fluorometric quantification and RNA purity was assessed by measurements of 260/280 nm and 260/230 nm ratios. To quantify the presence of PCR inhibitors, a qPCR assay was used in which the RNA eluate containing potential inhibitors was added into the qPCR reaction containing an internal amplification control. The presence of inhibitors in the RNA eluates was determined by comparing the C_T values of reactions containing RNA eluate and with controls that did not contain eluate. This assay was performed using the QuantiFast® Pathogen +IC kit.

Experiment 2: Isolation of DNA and RNA from human stool samples using the RNeasy PowerMicrobiome Kit, IRT protocol, on the QIAcube.

Total nucleic acids were isolated from human stool samples using either the manual protocol or the QIAcube IRT protocol with the RNeasy PowerMicrobiome Kit. For both protocols 200 mg from the same human stool sample were placed into PowerBead Tubes. Cells were lysed according to the kit manual by adding 650 µl Solution PM1 with β-mercaptoethanol and 100 µl phenol/chloroform/isoamylalcohol, followed by vortexing for 10 minutes at maximum speed using a 24-sample vortex adapter. To allow for a reliable comparison between manual and automated

sample processing, supernatants were pooled after mechanical lysis. This minimizes the impact of sample-to-sample variation when working with a heterogeneous sample material such as stool. From this pool of supernatants, 450 µl of lysate per sample was then processed with the manual and QIAcube protocols according to instructions in the *RNeasy PowerMicrobiome Kit Handbook*. Briefly, for the manual protocol, after mechanical lysis IRT was used to remove contaminants from the stool sample, followed by binding of DNA and RNA to a silica-based spin column and washing and elution in 100 µl of RNase-free water. For the QIAcube protocol, after mechanical lysis 450 µl per sample was transferred into position 2 of the rotor adapter which was then placed into the centrifuge of the QIAcube. All subsequent steps including the removal of inhibitory substances were performed on the QIAcube. For each protocol, 6 samples were processed. After isolation, DNA and RNA yield was determined by fluorometric quantification and nucleic acid quality was assessed by measurements of 260/280 nm and 260/230 nm ratios.

Experiment 3: Isolation of DNA and RNA from dog stool samples using the RNeasy PowerMicrobiome Kit, standard protocol, on the QIAcube.

Fecal samples of different origin can vary greatly in their chemical properties. To allow for maximum flexibility when working with the RNeasy PowerMicrobiome Kit on the QIAcube, there are protocols available that combine manual processing of samples for the removal of inhibitors and automated isolation of nucleic acids (standard protocol). By automating the bind, wash and elution steps, as well as on-column DNase digest, these protocols decrease hands-on time significantly. Total nucleic acids or only RNA were isolated from dog stool with a protocol that includes on-column DNase treatment. For this, 200 mg of dog stool were transferred into PowerBead Tubes and processed according to the kit handbook. After the addition of 650 µl Solution PM1 with β-mercaptoethanol and 100 µl phenol/chloroform/isoamylalcohol, samples were vortexed for 10 minutes at maximum speed using a 24-sample vortex adapter. Contaminants were removed from the samples by adding Solution IRS. Resulting supernatants were pooled to minimize the impact of sample-to-sample variation when comparing the manual and QIAcube protocols of the RNeasy PowerMicrobiome Kit. For manual extraction, 600 µl of sample were processed according to the handbook either with or without on-column DNase treatment. For the extraction on the QIAcube, 600 µl from the same supernatant pool were transferred into 2 ml safe lock tubes in the shaker of the QIAcube. All subsequent steps including DNase treatment were performed on the QIAcube. For the protocol without DNase digest, 10 samples were processed both manually and with the QIAcube. For the protocol with on-column DNase digest, 5 samples were processed with the manual and QIAcube protocols of the RNeasy PowerMicrobiome Kit. After isolation, DNA and RNA yield were determined by fluorometric quantification and nucleic acid quality was assessed by measurements of 260/280 nm and 260/230 nm ratios.

Results and discussion

Experiment 1: Comparable RNA yield and purity from human stool with manual and automated IRT protocols of the RNeasy PowerMicrobiome Kit, with on-column DNase digest.

The performance of the RNeasy PowerMicrobiome Kit protocol, with on-column DNase digest, was measured on samples processed using the QIAcube. Yield and purity of RNA isolated from human stool was compared for samples processed with the automated protocol to RNA isolated with the manual protocol. RNA isolated with the QIAcube version of the protocol had a comparable RNA yield and quality when compared with manual isolation (Figure 1).

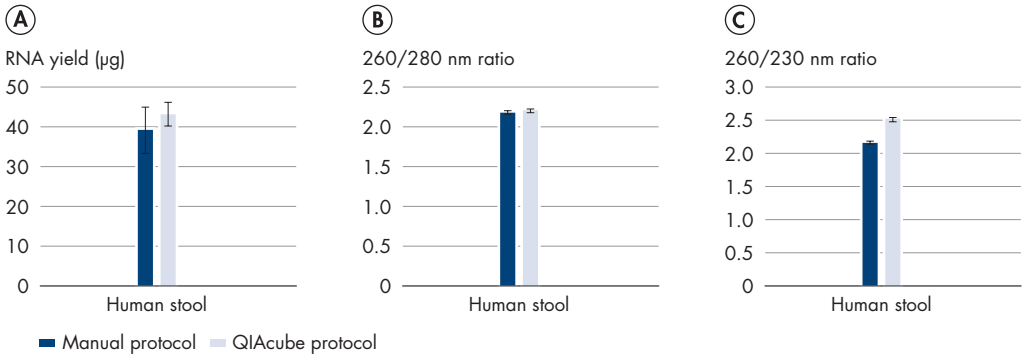

Figure 1. Comparable RNA yields between QIAcube and manual protocols of the RNeasy PowerMicrobiome protocols. **A** RNA yield was measured by a fluorometric-based, RNA specific assay. **B** and **C** RNA purity was determined by spectrophotometric measurements at 260 nm and 280 nm and at 260 nm and 230 nm. Displayed is the average of 6 samples per protocol. The standard deviation is shown for each condition.

A test for the efficient removal of small molecule PCR inhibitors which can interfere with enzymatic downstream applications was also performed. Quantitative PCR analysis confirmed that automated RNA isolated from human stool had similar or fewer inhibitors present in the eluates compared with manual isolation (Figure 2). In particular, the QIAcube protocol completely removed inhibitors from typically inhibitor-rich stool.

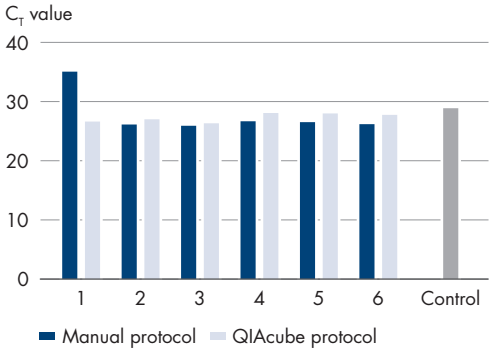

Figure 2. Efficient removal of PCR inhibitors was demonstrated by processing samples isolated with the manual and QIAcube RNeasy PowerMicrobiome Kit protocols with the QuantiFast Pathogen +IC Kit. 10 µl of eluate from each sample were added to reaction mixtures that contained internal control DNA. Amplification of this control DNA was quantified by qPCR. The C_T value of a water control which does not inhibit amplification of the internal control DNA was compared to samples containing eluates from the RNeasy PowerMicrobiome protocol. Eluates from the QIAcube protocol performed as well or better than the manual protocol as can be seen by a C_T value comparable to the control. Each vertical bar represents an individual sample.

Taken together, these data show that automation of the RNeasy PowerMicrobiome Kit, with on-column DNA digest, on the QIAcube performs equally well as the manual version of the kit. In addition, hands-on time for removal of inhibitory substances and spin column based DNA isolation is significantly reduced. The extracted DNA is high-quality and can be used immediately in downstream reactions, including next-generation sequencing and qPCR.

Experiment 2: Comparable RNA and DNA yield and quality from human stool with manual and automated IRT protocols of the RNeasy PowerMicrobiome Kit.

To measure the performance of the RNeasy PowerMicrobiome Kit protocol on the QIAcube, DNA and RNA yield and purity isolated from human stool samples using the automated protocol was compared with the manual protocol. Both DNA and RNA isolated with the QIAcube protocol had a similar yield and purity when compared with manual isolation (Figure 3). Therefore, the data shows that automation of the RNeasy PowerMicrobiome Kit on the QIAcube performs equally as well as the manual version of the kit while significantly reducing hands-on time for inhibitor removal and DNA/RNA purification. The high quality of the DNA and RNA and efficient removal of inhibitors allows for direct use in downstream analyses, including next-generation sequencing applications and qPCR assays.

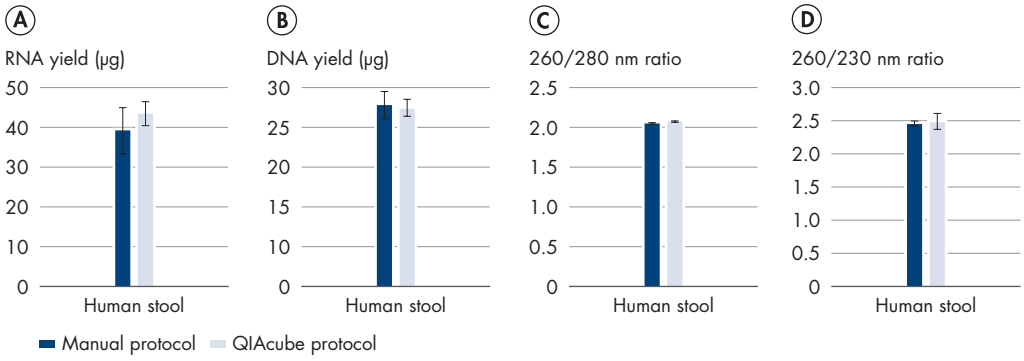

Figure 3. Comparable RNA and DNA yields between QIAcube and manual protocols with the RNeasy PowerMicrobiome Kit. **A** RNA yield and **B** DNA yield were measured by specific fluorometric based assays. **C** and **D** Total nucleic acid purity was determined by spectrophotometric measurements at 260 nm and 280 nm and at 260 nm and 230 nm. Displayed is the average of 6 samples per protocol. The standard deviation is shown for each condition. There were no significant differences between the QIAcube and manual protocols.

Experiment 3: Comparable DNA and RNA yield and purity from dog stool samples using the RNeasy PowerMicrobiome Kit, standard protocol, on the QIAcube.

To measure the performance of the RNeasy PowerMicrobiome Kit, standard protocol, on the QIAcube, DNA and RNA yield and purity isolated from dog stool using the automated protocol was compared to the manual protocol. For this, inhibitor removal was carried out manually followed by nucleic acid isolation on the QIAcube. Manual and automated protocols were ▷

compared with and without on-column DNase digest. Both RNA and DNA isolated with the QIAcube standard version of the protocol had a comparable yield and purity when compared with manual isolation (Figure 4).

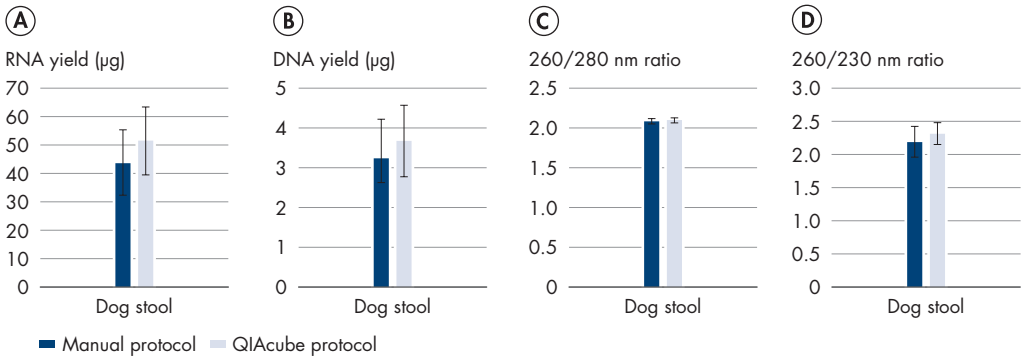

Figure 4. Comparable RNA and DNA yield and purity with the QIAcube standard and manual protocols of the RNeasy PowerMicrobiome Kit. A RNA yield and **B** DNA yield were measured by specific fluorometric based assays. **C** and **D** Total nucleic acid purity was determined by spectrophotometric measurements at 260 nm and 280 nm and at 260 nm and 230 nm. Displayed is the average of 10 samples per protocol. The standard deviation is shown for each condition. There were no significant differences between the QIAcube and manual protocols.

Next, the performance of the RNeasy PowerMicrobiome Kit, standard protocol, was measured including on-column DNase digestion on the QIAcube. RNA yield and quality isolated from human stool using the automated protocol was compared to RNA isolated with the manual protocol. RNA isolated with the QIAcube standard version of the protocol had a comparable RNA yield and quality to manual isolation (Figure 5).

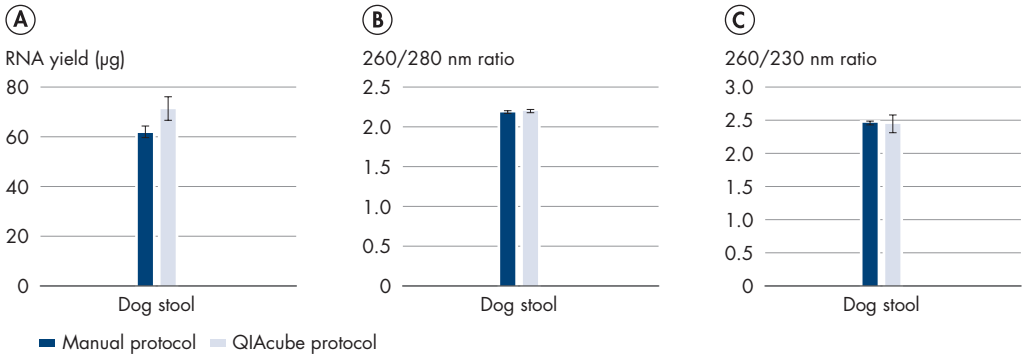

Figure 5. Comparable RNA yield and purity between the QIAcube standard protocol (including DNase digest) and manual protocol of the RNeasy PowerMicrobiome Kit. A RNA yield was measured by a RNA specific, fluorometric based assay. **B** and **C** RNA purity was determined by spectrophotometric measurements at 260 nm and 280 nm and at 260 nm and 230 nm. Displayed is the average of 10 samples per protocol. The standard deviation is shown for each condition. There were no significant differences between the QIAcube and manual protocols.

Taken together, these data show that automation of the RNeasy PowerMicrobiome Kit standard protocol, with and without DNase digestion, on the QIAcube performs equally well as the manual version of the kit while significantly reducing hands-on time for RNA/DNA purification. The possibility to carry out inhibitor removal manually combines flexibility with automation and allows processing of a wide range of sample types. The high quality of RNA and DNA, together with efficient removal of inhibitors allows for direct use in downstream analyses, including next-generation sequencing and qPCR assays.

Conclusions

- Automation of the RNeasy PowerMicrobiome Kit including on-column DNase digest allows efficient isolation of high-quality RNA from samples rich in inhibitors, such as stool samples, with minimal hands-on time.
- Automation of the RNeasy PowerMicrobiome Kit without on-column DNase digest is a convenient solution for simultaneous isolation of DNA and RNA from inhibitor-rich samples.
- The availability of a QIAcube protocol that includes automated inhibitor removal, a second protocol for manual removal of inhibitors with automated isolation of nucleic acids provides flexible options for a wide range of sample materials.
- Additional protocols for a range of sample materials with Inhibitor Removal Technology are available on the QIAcube, including the DNeasy® PowerSoil® Kit, DNeasy PowerLyzer® PowerSoil Kit, QIAamp® PowerFecal® Kit, DNeasy PowerPlant® Pro Kit and the DNeasy PowerClean® Pro Cleanup Kit.

Ordering Information

Product	Contents	Cat. no.
QIAcube	Robotic workstation for automated purification of DNA, RNA or proteins using QIAGEN spin-column kits: includes 1-year warranty on parts and labor	Inquire
RNeasy PowerMicrobiome Kit (50)	For the isolation of total RNA from stool and gut material	26000-50
PowerBead Tubes, Glass 0.1 mm (50)	Ready to use bead tubes for rapid and reliable biological sample lysis from a wide variety of starting materials	13118-50
Vortex Adapter for 24 (1.5–2.0 ml) tubes	For vortexing 1.7, 2, 5, 15 and 50 ml tubes using the Vortex-Genie® 2 Vortex	13000-V1-24
QIAamp PowerFecal DNA Kit	For the isolation of DNA from stool and gut material	12830-50

The QIAcube and all the kits mentioned here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease. The applications described here are not intended for diagnostic use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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