Application Note

RNA purification: A direct comparison of QIAcube® Connect with the Promega® Maxwell® RSC

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Introduction

Gene expression analysis allows the characterization of RNA transcripts present in a biological sample. However, it can be difficult to obtain sufficient quantities of RNA molecules for comprehensive gene expression analysis. QIAGEN offers dedicated kits for RNA purification for various tissue sample input amounts and elution volumes: the Rneasy® Plus Mini Kit and the RNeasy Plus Micro Kit.

The RNeasy Plus Micro Kit is designed for small amounts of cells (as little as 1 cell) or easy-to-lyse tissues. Suitable starting materials include laser microdissected (LMD) cryosections, fine-needle aspirates (FNA), and FACS®-sorted cells. The kit can be individually chosen depending on the sample type and input amount available. In addition, the desired concentration for accurate downstream analysis can be influenced by adjusting the elution volumes.

RNA purification from small samples requires elution in small volumes to achieve the highest RNA concentrations, for reliable downstream analysis. Automated sample purification increases the consistency of experiments and reduces operator bias, but the minimum elution volumes differ a lot between different automated purification technologies. The RNeasy Plus Mini Kit allows elution volumes of 30–100 µl; the RNeasy Plus Micro Kit provides an elution volume range of 14–30 µl on QIAcube Connect. Both kits – the RNeasy Plus Mini and the RNeasy Plus Micro – come with integrated gDNA removal that efficiently removes gDNA using a specially designed gDNA Eliminator spin column. The purified RNA is ready to use and is ideally suited for downstream applications that are sensitive to low amounts of DNA contamination, such as quantitative, real-time PCR.

QIAcube Connect fully automates the bind, wash and elute steps of more than 80 proven QIAGEN spin column kits and thereby allows very small elution volumes, compared to bead-based instruments. Here, we take the results of QIAcube Connect protocols for the RNeasy Plus Mini Kit and RNeasy Plus Micro Kit and compare them with results from the Maxwell RSC simplyRNA Tissue Kit protocol on the Maxwell RSC Instrument.

Materials and Methods

Different types of frozen, RNAprotect® Tissue Reagent–stabilized rat tissue were homogenized using the TissueRuptor®. A dilution series of the different homogenates was produced; RNA purification was performed using either the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC Instrument or the RNeasy Plus Mini Kit and the RNA Plus Micro Kit on the QIAcube Connect. For the RNeasy Plus

Micro Kit, 2.5 mg stabilized tissue from heart and kidney, as well as 5 mg frozen liver tissue, were used as starting material. For the RNeasy Plus Mini Kit, 10 mg stabilized tissue from heart and kidney, as well as 20 mg frozen liver tissue, were used as starting material. Equivalent starting amounts of the various tissue types were used with the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC instrument.

All experiments were performed in triplicate, and protocols were performed according to the manufacturer's instructions. RNA samples prepared with the RNeasy Plus Micro Kit were eluted in 15 µl, RNA samples prepared with the RNeasy Plus Mini Kit were eluted in 30 µl and RNA samples prepared with the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC were eluted in 50 µl. RNA concentration in the samples was determined using the RNA application of the QIAxpert® instrument.

RNA samples prepared with the Maxwell RSC simplyRNA Tissue Kit contained residual beads. Beads were removed from the RNA samples by magnetic separation using a DYNAL® magnet before further processing.

Efficiency of gDNA removal was determined using SYBR® Green–based quantitative real-time PCR. Analysis of 2 µl of the RNA eluates was performed using primers for pGK1. To check for residual gDNA in the RNA samples, RT control was performed in parallel. All reactions were run in triplicate on the Rotor-Gene® Q. Calculation of ΔCq was performed by subtracting the Cq-value of the reactions including RT from the Cq-value of the reactions without RT enzyme. Yields of the purified RNA were determined using the QIAxpert instrument. RNA integrity was checked using the 2100 Bioanalyzer® instrument.

Results

RNA yield and integrity

RNA yield and integrity were comparable for all purified RNA samples (data not shown).

RNA concentration

The RNeasy Plus Mini Kit and RNeasy Plus Micro Kit allow elution of the purified RNA in significantly lower elution volumes compared to the Maxwell RSC simplyRNA Tissue Kit. The lower elution volumes of the RNeasy Plus Mini Kit and RNeasy Plus Micro Kit resulted in significantly greater RNA concentrations of samples purified using the RNeasy Plus Mini and Micro Kits on QIAcube Connect (Figure 1 and Figure 2). RNA purity determined by A_{260}/A_{280} ratio was consistently good for all samples (data not shown).

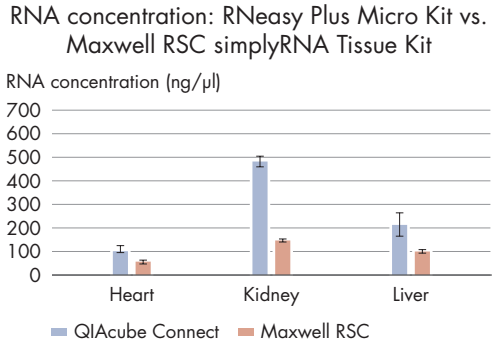

Figure 1. Greater RNA concentration in RNA samples prepared with the RNeasy Plus Micro Kit on QIAcube Connect. Automated RNA preparation using the RNeasy Plus Micro Kit on QIAcube Connect resulted in significantly higher RNA concentrations than when using the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC Instrument.

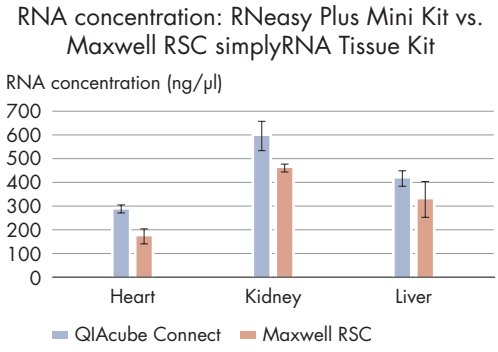

Figure 2. Greater RNA concentration in RNA samples prepared with the RNeasy Plus Mini Kit on QIAcube Connect. Automated RNA preparation using the RNeasy Plus Mini Kit on QIAcube Connect resulted in significantly higher RNA concentrations than when using the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC Instrument.

Volume of RNA eluates

The dead volume during the elution step of the RNA sample preparation method is critical for the recovery of the RNA from the samples. Higher dead volumes result in less RNA yield per elution and leads to irretrievable losses in yield. Analysis of the eluate volumes of the purified RNA samples demonstrated significantly lower dead volumes for the RNA samples prepared with the RNeasy Plus Micro Kit and RNeasy Plus Mini Kit on QIAcube Connect (Table 1).

Table 1. Elution, eluate and dead volumes of RNA samples prepared with the RNeasy Plus Micro Kit and the RNeasy Plus Mini Kit on QIAcube Connect and Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC

Method	Elution volume (µl)	Median eluate volume (µl)	Dead volume (%)
RNeasy Plus Micro Kit on QIAcube Connect	15	13	13
RNeasy Plus Mini Kit on QIAcube Connect	30	26	13
Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC	50	40	20

Efficiency of gDNA removal

Residual amounts of gDNA can add to the specific transcript signal in, e.g., quantitative qPCR analysis, leading to an overestimation of the gene expression profile. Elimination of genomic DNA is crucial for accurate gene expression results and when it's not possible to design RNA-specific primers or probes, for example, when analyzing single-exon genes.

The RNeasy Plus Mini and RNeasy Plus Micro Kits facilitate efficient gDNA removal with the unique gDNA Eliminator columns, without the need of DNase I digestion. Comparison of the gDNA removal efficiency shows significantly better gDNA removal with the RNeasy Plus Micro and RNeasy Plus Mini Kits (Figure 3 and Figure 4).

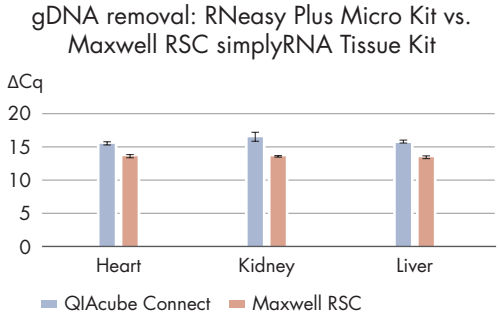

Figure 3. More efficient gDNA removal in RNA samples prepared with the RNeasy Plus Micro Kit on QIAcube Connect. Automated RNA preparation using the RNeasy Plus Micro Kit on QIAcube Connect resulted in higher ΔCq values, demonstrating a more efficient gDNA removal than in samples prepared with the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC Instrument.

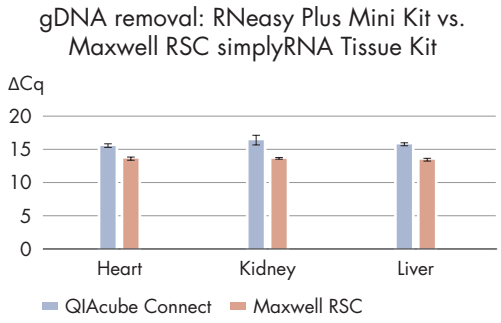

Figure 4. More efficient gDNA removal in RNA samples prepared with the RNeasy Plus Mini Kit on QIAcube Connect. Automated RNA preparation using the RNeasy Plus Mini Kit on QIAcube Connect resulted in significantly higher ΔCq values, demonstrating a more efficient gDNA removal than in samples prepared with the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC Instrument.

Conclusions

QIAcube Connect and the RNeasy Plus Mini and Micro Kits provided consistent, high-quality RNA purification for various tissue types, whether stabilized or frozen. The data shown here indicate significantly better performance of the RNeasy Plus Mini and Micro Kits run on QIAcube Connect with respect to RNA concentration, lower dead volumes during elution and gDNA removal.

In addition, automated sample prep on QIAcube Connect was more convenient compared to the RNA preparation on the Maxwell RSC. Samples purified with the Maxwell RSC simplyRNA Tissue Kit on the Maxwell RSC required an additional, tedious manual step to remove residual beads from the eluate.

The RNeasy Plus Mini Kit and RNeasy Plus Micro Kit allow elution of the purified RNA in significantly lower elution volumes compared to the Maxwell RSC simplyRNA Tissue Kit. This can result in slightly lower total RNA yields for some tissue types and starting amounts. If yield is of highest priority for the particular experiment, elution volumes can be increased or the elution step can be carried out twice.

Ordering Information

Product	Contents	Cat. no.
QIAcube Connect	For fully automated nucleic acid extraction with QIAGEN spin-column kits	9002864
RNAprotect Tissue Reagent	For immediate stabilization of RNA in tissues	76104
RNeasy Plus Micro Kit	Total RNA extraction from limited amounts of samples, with removal of gDNA	74034
RNeasy Plus Mini Kit	Total RNA extraction from limited amounts of samples, with removal of gDNA	74134

QIAcube Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications.

The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

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.