TECHNICAL NOTE

Automated RNA Isolation

Automated RNA purification from S-Monovette® RNA Exact from SARSTEDT with the chemagic™ 360 instrument

Abstract

Studies of transcriptome from whole blood are often limited due to the instability of RNA caused by nucleases and temperature after sample collection, storage, and a suboptimal isolation. In this Application Note, we show the successful stabilization of RNA by the S-Monovette® RNA Exact tubes from SARSTEDT in combination with automated RNA isolation with the chemagic™ Total RNA 9k Kit H24 (CMG-1084-S) on the chemagic™ 360 instrument. High-quality RNA was extracted after up to seven days stored at room temperature and up to 14 days stored at 4 °C.

Introduction

The study of RNA expression in whole blood opens a wide range of interesting research capabilities, but one limitation of studies using whole blood as a source is the RNA degradation during the collection from donors and subsequent sample storage by nucleases or inappropriate temperatures. RNA quality is essential as the RNA stability ultimately affects subsequent analysis of the transcriptome. The development and availability of blood collection tubes has simplified the RNA purification from whole blood samples enormously. Now, SARSTEDT has developed a new RNA blood collection tube called S-Monovette® RNA Exact (01.2048.001, SARSTEDT, Nümbrecht, Germany). The contained solution immediately lyses blood cells after sample collection and inhibits degradation and *de novo* synthesis of RNA. In this Application Note, we show the automated RNA purification with our chemagic™ Total RNA 9k Kit H24 using the chemagic™ 360 instrument from the S-Monovette® RNA Exact tubes and the quality of isolated RNA after several days of storage at different temperatures.

PerkinElmer®
For the Better

Product overview

PerkinElmer chemagen developed the chemagic™ Total RNA 9k Kit H24 to isolate RNA from 2.4 ml fresh and frozen blood stabilized in the S-Monovette® RNA Exact tubes with the chemagic™ 360 instrument (Figure 1).

The chemagic™ Total RNA 9k Kit H24 is based on the chemagen technology using M-PVA Magnetic Beads for the isolation of RNA. Nucleic acids bind to paramagnetic beads, which are magnetically separated from the sample material. During subsequent steps, contaminants are removed, DNA is digested, and the purified RNA is transferred into an elution medium.
The automated sample processing by the chemagic™ 360 instrument excludes cross contamination and ensures safe handling of infectious sample material.

For more details about the technology, visit
https://chemagen.com/technology/

Figure 1 chemagic™ 360 instrument equipped with the chemagic™ 24 Rod Head was used for RNA isolation from blood stored in S-Monovette® RNA Exact tubes.

Product Name	Kit Name	chemagic™ Rod Head	Format	Preps/Kit	Sample Volume	Hands-on time
CMG-1084-S	chemagic™ Total RNA 9k Kit H24	24	24-well	240	9 ml	10 min*

* After thawing of samples.

Materials and Methods

2.4 ml blood from five donors was drawn into the S-Monovette® RNA Exact tubes (01.2048.001, SARSTEDT, Nümbrecht, Germany). The collection tubes were stored for up to seven days at room temperature and for up to 14 days at 4 °C. Afterwards, the S-Monovette® RNA Exact tubes were stored at -80 °C. For RNA isolation, the collection tubes were thawed for 2 h at room temperature. The complete volume of blood and stabilizing solution of the S-Monovette® RNA Exact tubes (up to 9 ml) was used in the automated RNA extraction on a chemagic™ 360 with the chemagic™ Total RNA 9k Kit H24. The sample preparation with the chemagic™ Total RNA 9k Kit H24 does not involve any centrifugation or filtration, which leads to a total hands-on time of only 10 min.

The RNA quantity and purity were determined with UV measurement using an Epoch™ Microplate Spectrophotometer (BioTek Instruments, Winooski, Vermont, USA). In addition, the concentration and yield were measured with the Qubit™ RNA BR Assay Kit (Q10210, Thermo Fisher Scientific™, Waltham, Massachusetts, USA).

The RNA integrity (RNA quality score, RQS) was measured with the RNA Assay Reagent Kit (CLS960010, PerkinElmer, Hopkinton, Massachusetts, USA) and LabChip® DNA 5K/RNA/CZE (760435, PerkinElmer, Hopkinton, Massachusetts, USA) run on a LabChip® GX Touch HT (PerkinElmer, Hopkinton, Massachusetts, USA).

Reverse transcription (RT) of the purified RNA into cDNA was performed with the First Strand cDNA Synthesis Kit (#K1612, Life Technologies, Darmstadt, Germany) following the manufacturer's instructions. To avoid cDNA synthesis of fragmented mRNA without a Poly(A) tail, only the poly(T) primers were used for the cDNA synthesis. After cDNA synthesis, quantitative real-time PCR (qPCR) was performed with the Maxima SYBR® green/ROX qPCR Master Mix (#K0223, Thermo Fisher Scientific™ , Waltham, Massachusetts, USA) on a QuantStudio™ 5 (A34322, Thermo Fisher Scientific™, Waltham, Massachusetts, USA). The following five primer pairs were used for qPCR (Table 1). The primers were synthesized by biomers.net (Ulm, Germany).

Name	Gene and accession No.	Primer sequence (3‘ à 5‘)	Amplicon length
IL8	Chemokine ligand 8 NM_000584.3	IL8_fw: GGAAGGAACCATCTCACTGTG IL8_rv: GGAGTATGTCTTTATGCACTGAC	151 bp
IL1B	Interleukin 1, beta NM_000576.2	IL1B_fw: AAACCTCTTCGAGGCACAAGG IL1B_rv: GTCCTGGAAGGAGCACTTCATC	198 bp
FOS	FBJ murine osteosarcoma viral oncogene homolog NM_005252.3	FOS_fw: TCAACGCGCAGGACTTCTGC FOS_rv: TCTCCGCTTGGAGTGTATCAGTC	375 bp
FOXP3	Forkhead box P3 NM_014009.3	FOXP3_fw: AACAGCACATTCCCAGAGTTCC FOXP3_rv: GGATGGCGTTCTTCCAGGTGG	205 bp
TNFRSF10C	Tumor necrosis factor receptor superfamily member 10c NM_003841.3	TNFRSF10C_fw: ATCCCCAAGACCCTAAAGTTCG TNFRSF10C_rv: GAGATCCTGCTGGACACTCCTC	163 bp

Results

The concentration and yield are highly dependent on the donor. Yields obtained with five different donors varied between 2.5 - 10.5 µg RNA for samples stored at room temperature for up to seven days, and between 2 - 9.5 µg RNA for samples stored at 4 °C for up to 14 days. Qubit and UV-measurement of yields showed comparable results.

The RQS (RNA Quality Score) showed good results over the investigated time periods for both storage temperatures, either at 4 °C or at 22 °C (room temperature). Figure 2 shows the mean RQS obtained from the five different donors used in this study. According to Fleige et al. [1], RNA integrity above five is considered as good, while an RNA integrity above eight shows perfect RNA quality.

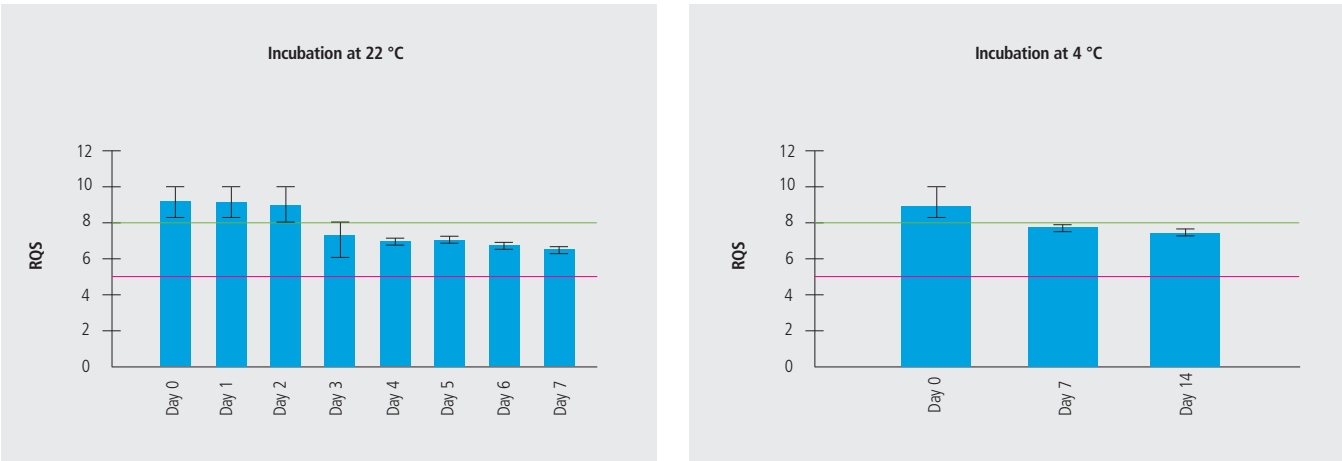

Figure 2 Excellent RNA integrity for five donors was obtained from isolated RNA stored in S-Monovette® RNA Exact tubes at 22 °C (left) and 4 °C (right). High-quality RNA (RQS > 8) was isolated after two days storage at 22 °C and even good RNA quality (RQS > 6) was obtained for up to seven days storage. Adequate RNA integrity was obtained for all tested time points over a period of 14 days at 4 °C.

To achieve efficient conservation of the gene transcription levels, it is required to stabilize the RNA after blood draw. Therefore, the stability of five different genes (FOS, FOXP3, IL1B, IL8 and TNFRSF10C) was tested via qPCR after cDNA synthesis. The qPCRs with all targets resulted in comparable C_T data, the data sets for FOS are shown exemplary in Figures 3 to 6.

To evaluate the effectiveness of the S-Monovette® RNA Exact tube to conserve RNA, the mean C_T values of RNA from all donors extracted from blood drawn in S-Monovette® RNA Exact tubes were compared with one competitor tube after incubation at room temperature for up to three days. Average C_T values reached with target FOS show a very good comparability between the S-Monovette® RNA Exact versus the competitor tube (Figure 3). After more than one day of incubation, even a slight trend towards lower C_T values could be observed with the S-Monovette® RNA Exact tube.

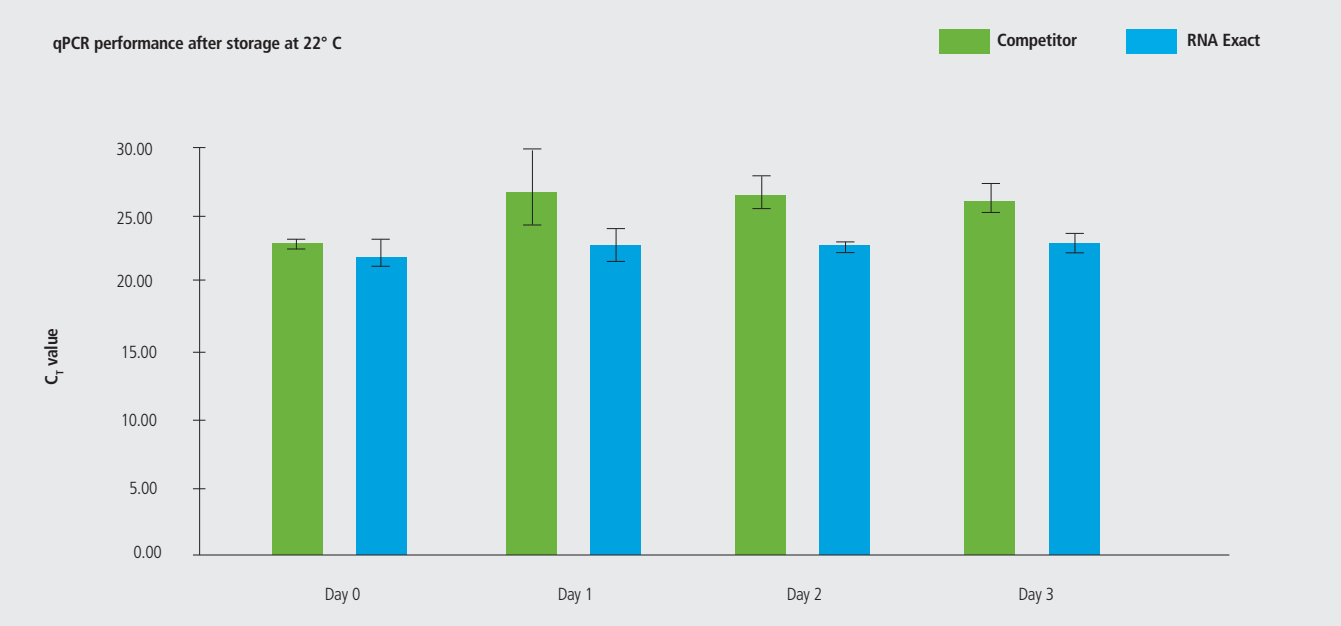

Figure 3 Comparison of qPCR performance for storage at 22° C. The isolated RNA from the S-Monovette® RNA Exact tubes demonstrates similar performance in qPCR compared to RNA isolated from a competitor tube after three days storage at room temperature. The C_T values obtained from RNA isolated from the S-Monovette® RNA Exact tubes were stable and slightly lower in comparison with the competitor tube indicating a slightly higher RNA yield.

Figure 4 Constant qPCR performance of RNA isolated from each single donor during 7 days of incubation at 22 °C. RNA isolated from RNA Exact tubes shows consistent C_T values over the measured time for all donors.

Further, the incubation time of the S-Monovette® RNA Exact tubes at 22°C was elongated to seven days (Figure 4) and likewise, incubation of the tubes at 4 °C was tested for up to 14 days (Figure 5). In all conditions, the C_T data of the five single donors are stable during the complete incubation time at both incubation temperatures.

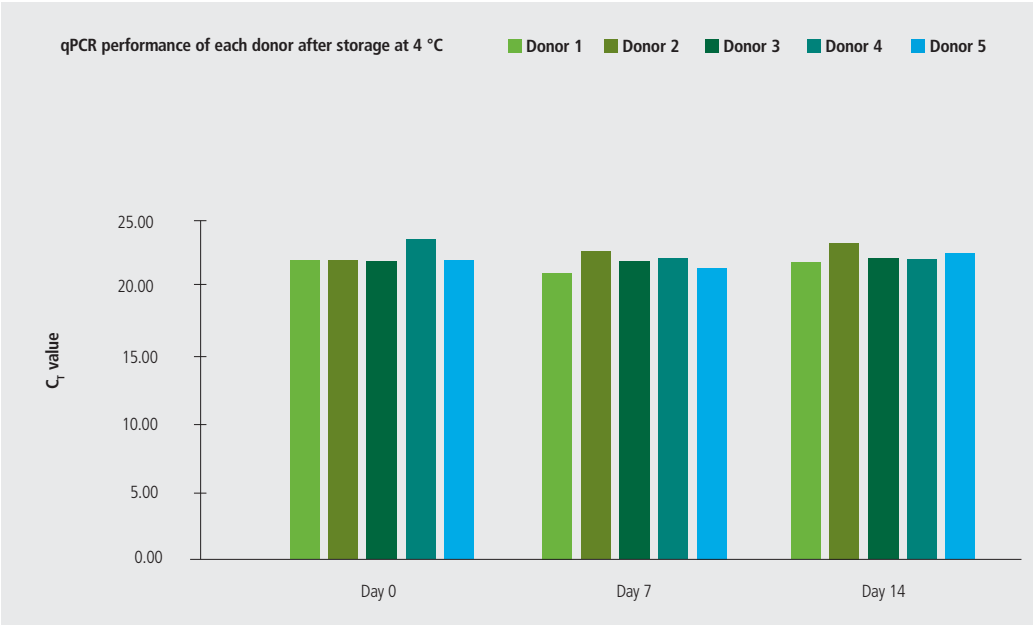

Figure 5 Consistent qPCR performance from RNA isolated from five single donors over 14 days of incubation at 4 °C. Nearly no C_T value difference was observed over 14 days of storage time, showing an extremely stable conservation of RNA collected with the S-Monovette® RNA Exact tubes and extracted with the Total RNA 9k Kit H24 (CMG-1084-S) on the chemagic™ 360 instrument.

PerkinElmer®
For the Better

Discussion

RNA yields obtained from the five donors were in a reasonable range compared to other publications [2, 3]. The RNA yields showed expected large variations, resulting from donor-specific differences in the white blood cell (WBC) counts as described in Chomczynski et al. [4]. The RQS from RNA isolated from stored blood samples incubated at 22 °C and 4 °C showed good results for RNA integrity over the tested incubation time after extraction with the chemagic™ Total RNA 9k Kit H24. Storage time at 22 °C and 4 °C is dramatically elongated with S-Monovette® RNA Exact tubes compared to other vendors of RNA tubes [5, 6]. The stabilization of RNA levels after blood draw was shown with five different primer sets in RT-qPCR. Compared to eluates extracted from a competitor tube, the C_T values were more stable over three days and showed no increase even after seven days at 22 °C or after 14 days at 4 °C.

In conclusion, the high-quality RNA isolated from blood stored in the S-Monovette® RNA Exact tubes performed excellently in all common downstream applications with the automated isolation procedure of chemagic™ Total RNA 9k Kit H24 on the chemagic™ 360 instrument. The S-Monovette® RNA Exact tubes offer several advantages in comparison to competitor tubes. The main advantage is a facilitated sample preparation without centrifugation steps, resulting in a significantly decreased hands-on time. Moreover, the elongated storage time of the S-Monovette® RNA Exact tubes enables more flexibility in scheduling RNA extraction and downstream assays.

In addition to the chemagic™ Total RNA 9k Kit H24, PerkinElmer chemagen provides further solutions for RNA extraction from blood collection tubes such as the chemagic™ RNA Blood 2.5k Kit H24 to be used on the chemagic™ 360 instrument equipped with a chemagic™ Rod Head 24 for processing of up to 24 samples per run (7). In addition, PerkinElmer provides the chemagic™ RNA Tissue 10mg Kit H96 for automated RNA isolation from tissue or white blood cell pellets on the chemagic™ 360 equipped with a chemagic™ Rod Head 96 to process up to 96 samples at once.

Literature

1. Fleige S, Pfaffl MW. RNA integrity and the effect on the real-time qRT-PCR performance. Molecular Aspects of Medicine. Apr-Jun;27(2-3):126-139 (2006). DOI: 10.1016/j.mam.2005.12.003. PMID: 16469371.
2. Aarem, J., Brunborg, G., Aas, K.K. et al. Comparison of blood RNA isolation methods from samples stabilized in Tempus tubes and stored at a large human biobank. BMC Res Notes 9, 430 (2016). doi.org/10.1186/s13104-016-2224-y
3. Gautam, Aarti et al. Investigating gene expression profiles of whole blood and peripheral blood mononuclear cells using multiple collection and processing methods. PloS one vol. 14,12 e0225137 (2019). doi:10.1371/journal.pone.0225137
4. Chomczynski P, Wilfinger WW, Eghbalnia HR, Kennedy A, Rymaszewski M, et al. Inter-Individual Differences in RNA Levels in Human Peripheral Blood. PLOS ONE 11(2): e0148260 (2016). doi.org/10.1371/journal.pone.0148260
5. https://www.qiagen.com/de/products/discovery-and-translational-research/sample-collection-stabilization/rna/paxgene-blood-rna-tubes/
6. https://www.thermofisher.com/order/catalog/product/4342792#/4342792
7. Herrera-Rivero, M., Zhang, R., Heilmann-Heimbach, S. et al. Circulating microRNAs are associated with Pulmonary Hypertension and Development of Chronic Lung Disease in Congenital Diaphragmatic Hernia. Sci Rep 8, 10735 (2018). doi.org/10.1038/s41598-018-29153-8

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer chemagen Technologie GmbH
Arnold-Sommerfeld-Ring 2
52499 Baesweiler, Germany
Phone: (+49) 2401 805-501
Fax: (+49) 2401 805-519
support.chemagen@perkinelmer.com, www.chemagen.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

AG012108_25_AP PKI