InviMag® Blood RNA Exact Kit/ IG & S-Monovette® RNA Exact

A Complete System for Collection, Stabilizing & Walk Away Purification of RNA from Whole Blood in Standardized Workflow

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Abstract

The InviMag® Blood RNA Exact Kit/ IG in combination with the InviGenius® PLUS robotic platform is a novel fully automated and standardized extraction tool for total RNA from whole blood stabilized in the S-Monovette® RNA Exact Tube with very convenient handling.

Introduction

Gene expression profiling and analysis based on peripheral whole blood is becoming increasingly important in life science and molecular diagnostics (1). Accurately measuring the molecular profile of blood-based RNA transcripts plays an important role in areas such as cancer research, biomarker discovery, target validation, monitoring drug treatment regimes and to predict genetic risks of diseases (2).

RNA levels in blood are affected by the highly unstable nature of RNA transcripts *ex vivo* (3,4). Ongoing metabolic changes can drastically affect gene expression profiles in timeframes as short as one hour, so blood storage conditions can significantly alter gene expression results (4,5).

To solve this problem, SARSTEDT has developed the S-Monovette® RNA Exact for the direct collection of 2.4 ml of human whole blood into a blood collection tube (polypropylene) containing an RNA preservation reagent, while maintaining the RNA expression or transcript profile and integrity. The RNA levels in whole blood samples are preserved for up to 5 days at room temperature (18 - 22°C) and up to 14 days at 2 to 8°C or for long-term storage at -40°C to -80°C. Transport can take place at ambient temperature or frozen for longer transport or storage.

InviMag® Blood RNA Exact Kit/ IG

To create a standardized workflow Invitek has developed the InviMag® Blood RNA Exact Kit/ IG using the InviGenius® PLUS for an automated high quality RNA preparation from blood collected in the S-Monovette® RNA Exact. The InviMag® Blood RNA Exact Kit/ IG workflow on the InviGenius® is easy to perform and can isolate RNA from up to 12 samples shorter than three hours starting from the complete stabilized sample.

Material and Methods

Blood samples from four donors were tested. Blood (2.7 ml each) was drawn into the S-Monovette® RNA Exact (#01.2048.001, SARSTEDT, Nümbrecht, Germany) and incubated for up to 10 days at 22,5 °C and at 8°C. Samples were frozen at – 80°C until isolation. For each donor the extraction was done at day D0, D1, D3, D5, D7 and D10.

After thawing the blood sample, RNA was isolated using the InviMag® Blood RNA Exact Kit/ IG on the InviGenius® PLUS platform following the manufacturer's protocol. After arranging of all kit components on the robotic platform, scanning of the sample barcode, the whole sample volume of the blood RNA collection and stabilization tube (2.4 ml blood + 7.3 ml stabilization reagent) was added into the working plate, which was placed on the InviGenius® PLUS surface. The fully automated purification process was started. The hands-on time is less than 30 min and does not require any centrifugation or filtration step as compared to leading competitor's process. Afterwards the purified total RNA was eluted into strips or screw tubes.

For all samples following analysis were done: RNA yield, concentration and purity (OD A260/A280 – OD A260/A230) was measured with a Nanodrop 1000 Photometer (Thermo). RNA integrity was measured by Bioanalyzer 2100 (Agilent, Waldbronn, Germany) using RNA Nano 6000 Chips (Agilent). The content of DNA in the eluted RNA was analysed by qPCR using the InviQuant GeneCount 40 Kit (# 3130100100 Invitek Molecular GmbH, Berlin, Germany).

Purified RNA was reverse transcribed into cDNA using First Strand cDNA Synthesis kit (#K1612, Life Technologies, Darmstadt, Germany) according to manufacturer's instructions.

All RT-qPCRs were performed with Maxima SYBR Green/ROX qPCR Master Mix (#K0223 Thermo Fisher Scientific, Waltham Massachusetts, USA) according to manufacturer's protocol on a Master cycler ep realplex 4s (Eppendorf, Hamburg, Germany). Primers were used in final concentration of 0,5 µM. After an initial denaturation step of 10 min at 95°C, 40 cycles of denaturation (15 sec. at 95°C) annealing (15 sec. at 59°C) and elongation (15 sec. at 68°C) were performed.

Results

1. RNA yield

Blood from four donors was collected in S-Monovette® RNA Exact tubes and stored for up to 10 days at 22,5 °C and at 8°C. Samples were frozen at – 80°C until isolation. RNA from samples stored at 22,5°C was isolated at day D0, D1, D3, D5, D7 and D10 and samples stored at 8°C were isolated at day D0, D3; D7 and D10 using the InviMag® Blood RNA Exact Kit/ IG on the InviGenius® PLUS. High yields of high purity RNA could be isolated reproducebly, regardless of the storage temperature and storage time of the blood in the S-Monovette® RNA Exact.

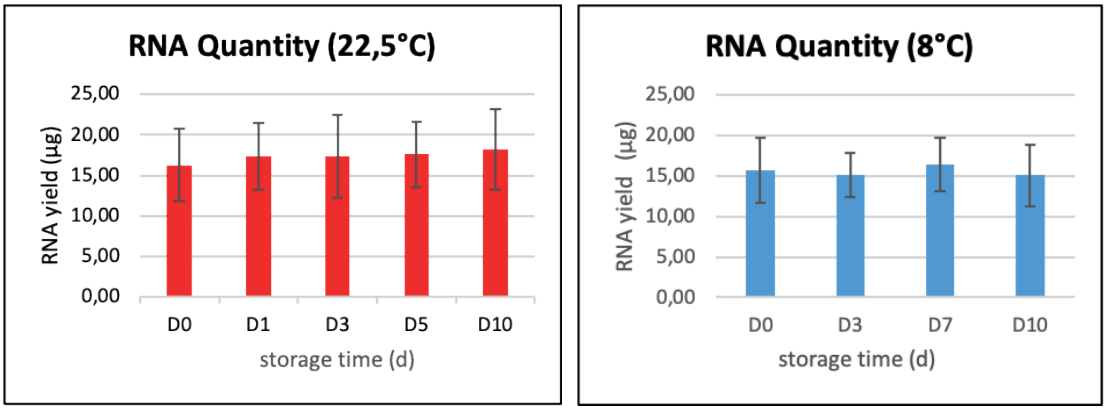


Figure 1: RNA quantity and stability from blood samples stored at 22.5 °C and 8°C

2. RNA integrity

Integrity of RNA purified from blood stored *in situ* in S-Monovette® RNA Exact tubes at 22.5°C and 8°C was consistently very good. Mean RNA integrity number (RIN) scores for 4 specimens are given for the indicated storage times (D0-D5 respectively D0 - D10), shown in figure 2.

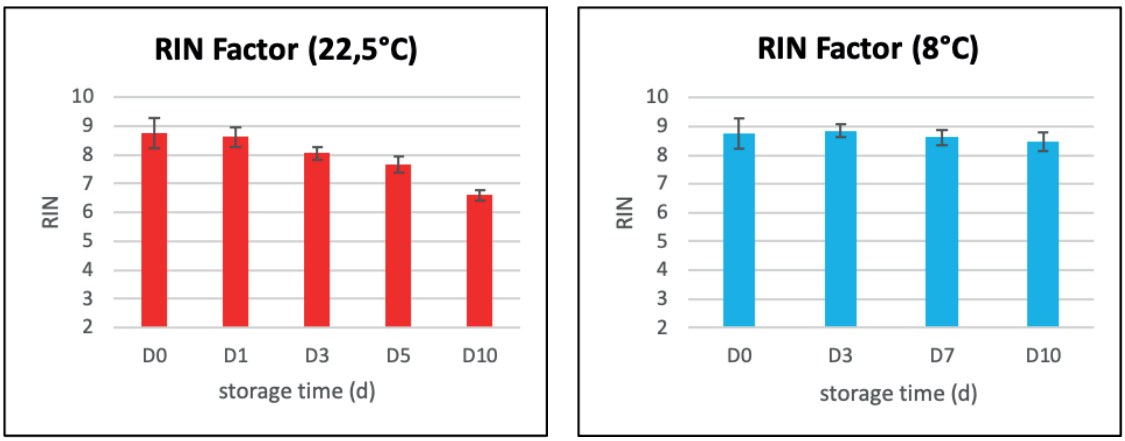
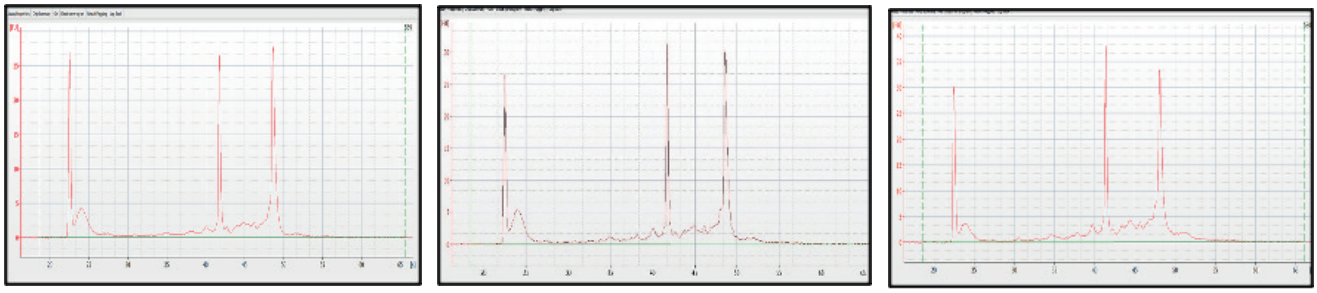


Figure 2: RNA integrity in stored blood samples (storage temperature: 22.5 °C and 8°C)

The error bars indicate the standard deviations of RIN scores for the 4 specimens. The values D0, D3, D7 and D10 show no significant differences at 8°C, as expected. The values D3 and D5 for the 22.5°C series are minimally reduced within the specifications (RNA of high quality with a RIN >7.5), while D1 is in the same range as the D0 control. Only D10 (22.5°C) which is out of storage specifications, shows a significant reduction of RIN.
The high quality and integrity of the total isolated RNA (Figure 3) is documented by the two ribosomal RNA peaks (18s-rRNA; 28s-rRNA), as well as the flat baseline between the internal marker and the 18s-rRNA peak in the electropherogram. Furthermore, there is no evidence of degradation products of rRNA.



Donor 126; day 0 | *Donor 126; day 3; stored at 22,5°C* | *Donor 126; day 10; stored at 8°C*

Figure 3: Bioanalyzer electropherogram of total RNA from one donor stored at different temperature and for different time schedules.

**All obtained data were collected in a cooperative validation study between Sarstedt and Invitek Molecular. In this study, the system was loaded with blood in the highest possible volume of 2.7 ml, while the standard median is 2.4 ml, this was done to show the robustness of the stabilization system. The difference in yield of RNA between 2.7 ml of blood and 2.4 ml of blood is negligible.*

3. RNA purity

RNA yield, concentration and purity (OD A_{260}/A_{280}, OD A_{260}/A_{230}) was measured with a Nanodrop 1000 photometer. With the help of the InviMag® Blood RNA Exact Kit/ IG in combination with the InviGenius® PLUS, high-purity, protein-free RNA was isolated from S-Monovette® RNA Exact stabilized whole blood, which is supported by the average ratio A_{260}/A_{280} of 2.1 over all RNA isolates. The ratio A_{260}/A_{230} is also 2.1 and also proves that the RNA isolate is free of further impurities (salts, phenol).

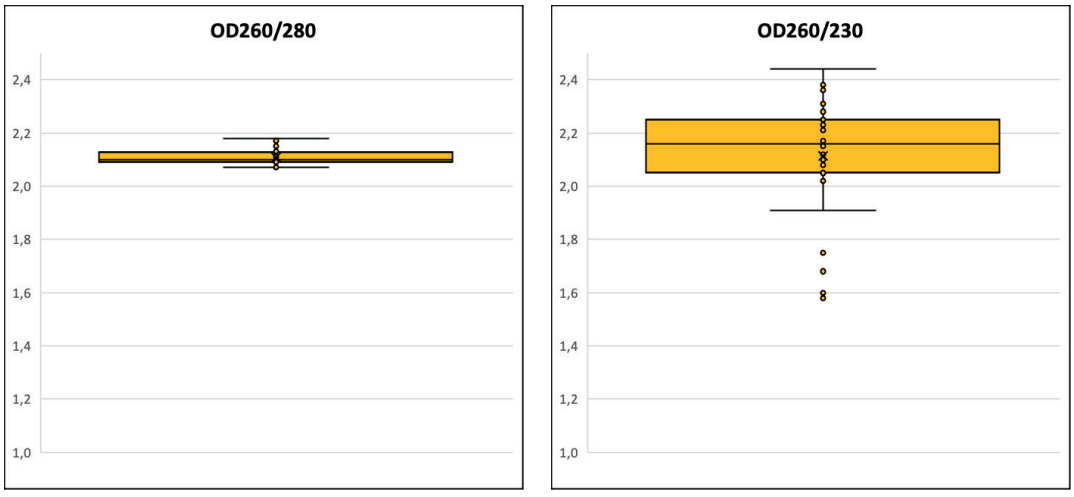


Figure 4: Nanodrop analyses over all samples and storage temperatures

4. DNA content

Samples shown in Figure 5 were subjected to genomic DNA content analysis in the eluted RNA using real time quantitative PCR of multi-copy target using the the InviQuant GeneCount 40 Kit. Genomic DNA was quantified using a standard curve method. Amounts of genomic DNA were related to the total nucleic acid content and the calculated percentage of genomic DNA contamination are shown as average amount over all samples and standard deviations (black bars) for all samples at different storage temperatures and storage times. The average DNA content over all samples and storage temperatures is ≤ 0,1 %.

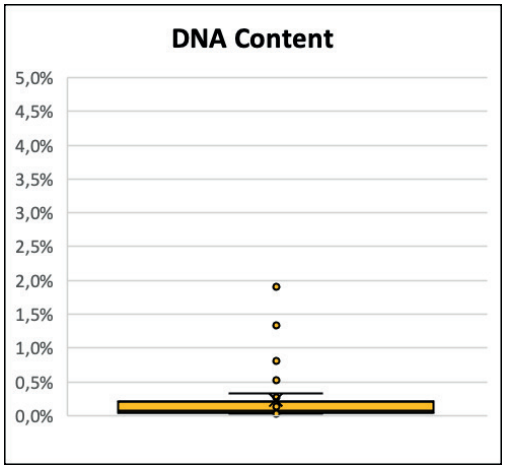


Figure 5: DNA contamination in % in the eluted RNA over all RNA samples

5. Transcript level analysis

The quality of isolated RNA was also tested by its usability for RT-qPCR. After isolation of total RNA from S-Monovette® RNA Exact stabilized whole blood, cDNA was synthetized and the expression levels of two housekeeping genes (*GNB2L1, PBGD*) were assayed, and used to normalize expression of four genes of interest (*IL8, Fos, IL1B, TNF*).

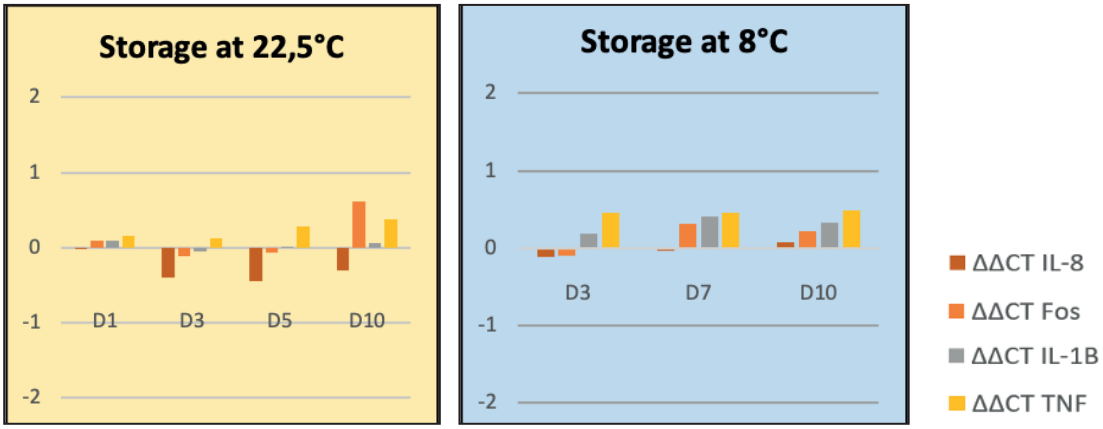


Figure 6: Stabilization of RNA conserves the expression level of genes at 22.5°C and at 8°C

The average values of 4 donors are presented in figure 6. All RNA samples (gene transcripts) were suitable for RT-qPCR analysis. Expression level was analysed after 1, 3, 5, 7, and 10 days' storage at determined temperature conditions. Extracted RNA expressions were compared via ΔC_T of 4 target genes (*IL-8, Fos, IL-1B, TNF*) in comparison to two housekeeping genes (*GNB2L1, PBGD*). $\Delta\Delta C_T$ values were calculated to monitor RNA level changes between the different storage times and the starting point D0, to prove RNA type independent stabilization. All 4 Genes showed $\Delta\Delta C_T$ values of <1, this shows that RNA is stabilized independently from respective gene-type.

Summary

The combination of the S-Monovette® RNA Exact and InviMag® Blood RNA Exact Kit/ IG on the InviGenius® PLUS platform provides an integrated system for collection, stabilization and an efficient and standardized automated extraction of intracellular RNA.

The system provides RNA stabilization for up to 5 days at 22 °C and for up to 14 days at 2 - 8°C. All transcript levels are maintained during the predicted times of stabilization at 22.5°C, as well as at 8°C. The system provides high yields of purified RNA (5 - 20 µg from full blood draw from normal, healthy donors), and high quality RNA (A_{260}: A_{280} ratio >1.9, and RIN factor between 7.5 - 9 at 22.5°C storage, and between 8 - 9 at 8°C storage), suitable for downstream applications, such as quantitative PCR, microarray profiling, and other RNA analysis methods.

Parameter	InviMag® Blood RNA Exact Kit/ IG (for 12 samples in parallel)
Procedure	automated extraction process InviMag® Blood RNA Exact Kit / IG
Processing Time	3.0 h in total
Sample Volume	2.4ml Blood in 7.3 ml stabilization solution of the S-Monovette® RNA Exact
Hands On Time	max. 0.5 h
Needed DNase I volume	2 µl / sample
Elution Volume	200 µl
Yield	≥ 5 - 20 µg
RNA Concentration	≥ 25 – 100 ng/µl
DNA Contamination	≤ 0.3 %
Ratio A_{260}:A_{280}	≈2,0
Ratio A_{260}:A_{230}	≈2,0
RNA Integrity	7 – 9
Purification Principle	Magnetic beads

References

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Ordering Information	Catalogue No.
InviMag® Blood RNA Exact Kit/ IG	2463320100
S-Monovette® RNA Exact (SARSTEDT)	01.2048.001
InviGenius® PLUS	5011100000