High-throughput purification of RNA from preserved RNAgard® blood samples using the MACHEREY-NAGEL NucleoSpin® 96 RNA Blood kit on a vacuum manifold.

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Abstract

In this application note we show the manual high-throughput extraction of RNA from blood samples preserved with the Biomatrica VACUETTE® RNAgard® Blood Tube. After three weeks of storage at 4 °C, RNA was extracted from human blood samples using the MACHEREY-NAGEL NucleoSpin® 96 RNA Blood kit on the NucleoVac Vacuum Manifold. RNA quality was subsequently assayed on the Agilent 2100 Bioanalyzer and in probe-based quantitative RT-PCR experiments.

Introduction

In gene expression studies the purification of RNA is a central process. Here the silica-based purification technology of the NucleoSpin® 96 RNA Blood kit enables a quick and simple way to process large sample numbers. However, RNA is very unstable in clinical sample materials such as whole blood and is quickly degraded by ribonucleases or through poor storage conditions. Therefore, in the past it was mandatory that the place were the samples were taken and the laboratory extracting the nucleic acids were in close vicinity to assure that very little time passed between sample-collection and extraction. To solve this problem, Biomatrica has developed the RNAgard® blood preservative reagent which is distributed prefilled in VACUETTE® blood collecting tubes. These tubes can be used to directly draw blood from the patient's vein using standard procedures. Here we show that high quality RNA can be extracted from RNAgard® blood samples by using the MACHEREY-NAGEL NucleoSpin® 96 RNA Blood kit. The resulting RNA is suitable for any subsequent test such as quantitative RT-PCR.

Material and Methods

- VACUETTE® RNAgard® Blood Tube (Biomatrica)
- Precipitation Buffer (Biomatrica)
- NucleoSpin® 96 RNA Blood kit (MACHEREY-NAGEL)
- NucleoVac Vacuum Manifold with accessories (MACHEREY-NAGEL)

For testing VACUETTE® RNAgard® Blood Tube compatibility with NucleoSpin® 96 RNA Blood kit, six blood samples have been tested. After the blood samples (2.5 mL) have been taken, the VACUETTES® were immediately mixed thoroughly to ensure the quick lysis of cells and the efficient RNA preservation.

After 20 days of storage at 4 °C, the samples were equilibrated to room temperature for 2 hours. The contents of the VACUETTES® were mixed and poured into a 50 mL conical tube. 3 mL of the Precipitation Buffer were added and the contents were mixed by vortexing. The tubes were then incubated at room temperature for 15 minutes on a rolling shaker. Afterwards, tubes were vortexed thoroughly and centrifuged at 5000 x *g* for 30 minutes in a swing bucket centrifuge.

Supernatant was decanted and the tubes were stood upside down on a tissue paper to remove the majority of the supernatant. A translucent red pellet was visible (Figure 1.)

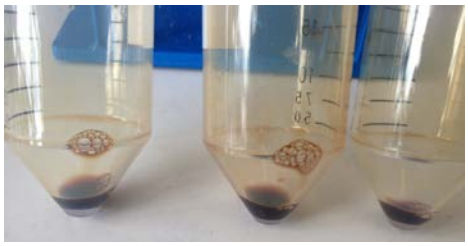


Figure 1: Precipitation pellet of human blood samples after addition of Precipitation Buffer to each conical tube containing the blood samples.

For three samples the pellet was re-dissolved in 800 µL (sample 1–3) and for the other three in 1600 µL (sample 4–6) of Buffer DL, respectively. For sample 4–6, 800 µL have been used for further processing. The samples were transferred to a 96-well Square-well Block and to each sample 20 µL of liquid Proteinase K were added. The samples were then incubated at ambient room temperature with shaking at 1200 rpm for 15 minutes.

Afterwards, 800 µL of Buffer RB4 were added to each sample. Samples were mixed by pipetting and loaded onto the NucleoSpin® 96 RNA Blood plate in two aliquots. A pressure of -0.2 bar was applied each time to load the RNA onto the silica membrane. The next steps were performed according to the MACHEREY-NAGEL NucleoSpin® 96 RNA Blood manual with standard buffer volumes.

Eventually the RNA was eluted in 75 µL of RNase-free water.

Results

As general note it is to mention that after the purification procedure there were virtually no residual precipitates left on the silica membranes of the NucleoSpin® RNA Blood Binding Plate (Figure 2.)

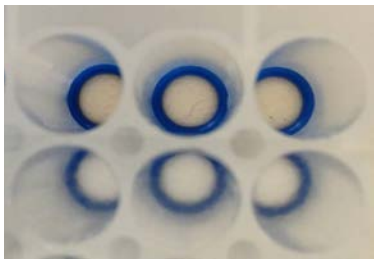
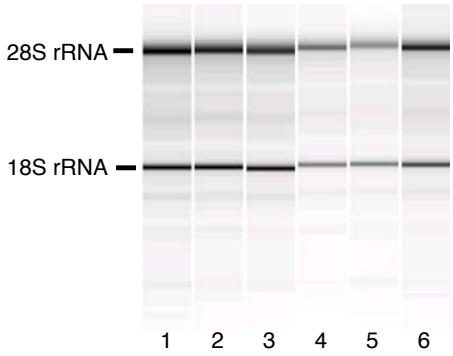


Figure 2: Clean silica-membrane of the NucleoSpin® 96 RNA Blood kit after sample loading. No sample or cell residues and precipitates are visible.

The quality of the obtained RNA was assayed on an Agilent 2100 Bioanalyzer using the RNA 6000 Nano kit. Figure 3A and 3B are showing high quality RNA without degradation and high RNA integrity. The mean RNA integrity number (RIN) was 8.4 for the complete samples with the lowest RIN being 7.8.

A



B

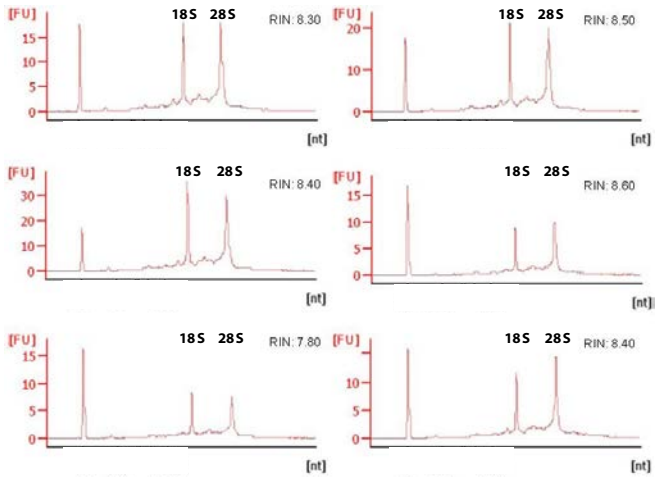


Figure 3: High RNA quality. Extracted RNA was assayed on an Agilent 2100 Bioanalyzer RNA 6000 Nano kit (A) and shows a high RNA integrity (B).

The average RNA concentration from 2.5 mL human blood samples was 85 ng/µL, which adds up to about 5 µg of pure RNA in 60 µL eluate.

For testing downstream performance, the extracted RNA samples were further evaluated by a probe-based qRT-PCR for ß-Actin.

Figure 4 shows that PCR is not inhibited. The obtained CT-values show only very little variation for identical samples. Yield is proportional to the used sample amount.

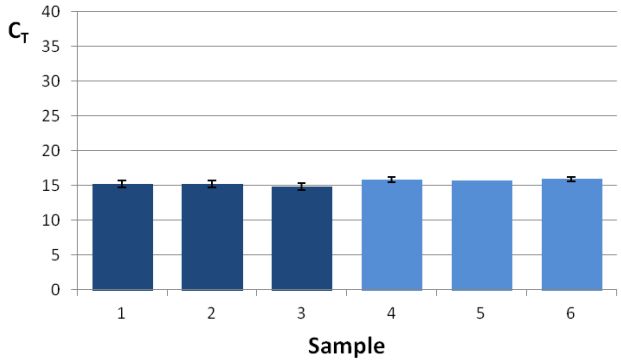


Figure 4: Real-time RT-PCR analysis of extracted RNA. Low C_T values indicate no PCR inhibition. Real-time RT-PCR was run on an ABI 7500 Real-Time PCR System and ß-Actin as reference gene.

Summary

Biomatrica's VACUETTE® RNAgard® Blood Tube provides a very good possibility to preserve RNA in blood samples. RNA is stable for at least 20 days when stored at 4 °C.

The NucleoSpin® 96 RNA Blood Kit from MACHEREY-NAGEL enables a quick and easy extraction of the preserved RNA. Extracted RNA is of high integrity and is optimal for downstream applications such as quantitative reverse-transcriptase PCR.

References

Application support

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Ordering information

Product	Preps	REF
NucleoSpin® 96 RNA Blood	2 x 96 4 x 96	740225.2 740225.4

For more information regarding the automated use of MN products, please contact your local representative or visit MN directly under ***www.mn-net.com***.