

Rapid Isolation and Purification of Total Leukocyte (White Blood Cell) RNA from Mammalian Blood Samples

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INTRODUCTION

Blood contains a great deal of cellular information that can be used to diagnose and treat human disease. Molecular markers of infection, inflammation, and autoimmune disease are carried in circulating leukocytes, which include the T- and B-lymphocytes, neutrophils, eosinophils, basophils, monocytes, and other less abundant cell types. Expression profiling tools, such as microarrays and real-time quantitative RT-PCR (qRT-PCR), provide a powerful means for identifying blood-based RNA biomarkers associated with pathological conditions (1, 2, 3). However, the sensitivity of expression profiling methodology for detecting leukocyte-derived mRNAs of interest is diminished by the large proportion of reticulocyte-derived globin mRNA present in RNA prepared from whole blood.

Based on the above information, Norgen has developed the Leukocyte RNA Purification Kit. Norgen's Leukocyte RNA Purification Kit provides a rapid method for the isolation and purification of total leukocyte RNA from mammalian blood samples (**Figure 1**). In this procedure, the red blood cells are first removed from the sample through differential red blood cell lysis, and the leukocytes are recovered through centrifugation. The leukocytes are lysed, and purification of the leukocyte RNA is then based on spin column chromatography using Norgen's proprietary resin as the separation matrix. Norgen's resin binds RNA in a manner that depends on ionic concentrations, and the RNA is preferentially purified from the other cellular components such as genomic DNA and proteins without the use of phenol or chloroform. The kit is able to isolate total leukocyte RNA, including all small RNA species, and the purified RNA is of the highest quality and can be used in a number of downstream applications.

This application note describes a method of isolating high quality leukocyte RNA using Norgen's Leukocyte RNA Purification Kit. Various parameters, including reproducibility and consistency of RNA quantity and quality, as well as the speed of the purification process, are analyzed.

MATERIALS AND METHODS

Leukocyte RNA Isolation

Total leukocyte RNA was isolated from whole hamster blood using Norgen's Leukocyte RNA Purification kit according to the provided protocol (**Figure 1**). Briefly, 5 volumes of RBC Lysis Buffer was added to a blood sample collected with EDTA and incubated at room temperature for 3 to 5 minutes. During the incubation the sample was vortexed several times to aid in mixing. The sample was then centrifuged at 250 x g for 3 minutes and the supernatant decanted. Two additional volumes of the RBC Lysis Buffer was then added to the pelleted white blood cells and mixed by gentle vortexing for 10 seconds. The tubes were again centrifuged at 250 x g for 3 minutes and the supernatant decanted. Next, 350 µL of Binding Solution was added to the pelleted leukocytes and the cells lysed by gentle vortexing until homogeneity was reached. To this mixture, 200 µL of 95-100% ethanol was then added. The mixture was vortexed for 10 seconds and the lysate applied onto an assembled spin column. The columns were centrifuged for 1 minute, the flowthrough discarded and the spin columns reassembled. The columns were then washed a total of three times using 400 µL of Wash Solution for each wash. The columns were then dried by spinning for 2 minutes, transferred to 1.7 mL elution tubes and the leukocyte RNA was eluted by adding 50 µL of Elution Buffer to the column and centrifuging for 2 minutes at 200 x g, followed by a 1 minute spin at 14,000 x g. In addition, RNA was isolated from 200 µL of whole hamster blood using the leading market competitor's kit according to the manufacturer's protocol for comparative analyses.

RNA Gel Electrophoresis

The purified RNAs were run on 1X MOPS, 1.2% formaldehyde-agarose gels for visual inspection. In general, 5 µL of each 50 µL elution was run on the gel.

Capillary Electrophoresis

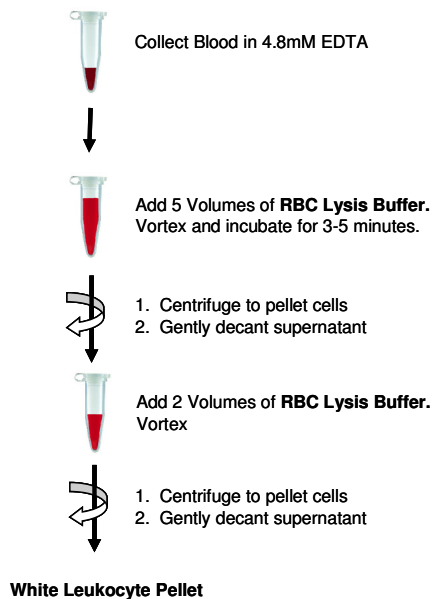
Purified RNAs from hamster blood were loaded onto an Agilent[®] RNA Nano 6000 chip and resolved on an Agilent[®] 2100 BioAnalyzer according to the manufacturer's instructions.

RT-qPCR Assays

RNA purified from hamster blood leukocytes was used as the template in an RT-qPCR reaction using primers specific for the *β-actin* gene or *miR-21*. First-strand cDNA synthesis was performed using Invitrogen's Superscript III system and the *miR-21* stem-loop RT primer or oligo dT.

The cDNAs were then used as the template in qPCR reactions using primers specific for *miR-21* or β -*actin*. Total RNA purified using the leading market competitor's kit was also used as template in the RT-qPCR reactions using primers specific for β -*actin* and *miR-21* for comparative analyses.

Flowchart 1: Procedure for Differential Red Blood Cell Lysis



Flowchart 2: Procedure for Leukocyte RNA Purification

Lyse leukocyte pellet using **Binding Solution**

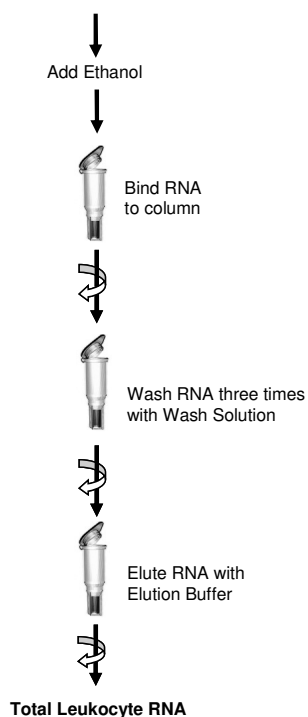


Figure 1. Procedure for the Purification of Total Leukocyte RNA using Norgen's Kit. Flow chart 1 demonstrates the steps involved in the differential red blood cell (RBC) lysis. Flow chart 2 demonstrates the procedure for the purification of total leukocyte RNA.

RESULTS AND DISCUSSION

RNA was isolated from 200 μ L of whole hamster blood using Norgen's Leukocyte RNA Purification Kit according to the provided protocol as described in **Figure 1**. RNA samples were initially run on a 1X MOPS, 1.2% formaldehyde-agarose gel for visual inspection (**Figure 2**). The purified RNA samples were of a high quality, with good yield and size diversity. Norgen's kit allowed for the consistent isolation of high quality RNA from all six replicates.

The quality of RNAs isolated by Norgen's Leukocyte RNA Purification Kit was further demonstrated by capillary gel electrophoresis (**Figure 3**). When the RNAs were resolved on an Agilent Lab-on-a-Chip, all the RNA species, including microRNA, 18S rRNA and 28S rRNA could be observed (Panel A). In contrast, the leading competitor's kit failed to purify the small RNA species. More importantly, Norgen's kit produced an RNA sample with a higher yield and purity than the leading market competitor's kit (Panel B). This is indicated by the lack of an additional peak between the two major rRNA bands in the Norgen sample. In contrast, the competitor sample had the additional peak between the rRNA bands as a result of RNA degradation. The results in Figure 3 demonstrate the compatibility of the small RNAs purified using Norgen's kit with the Bioanalyzer. This provides added benefits for quantifying the isolated microRNA, as spectrophotometry may not be applicable for the quantification of small RNA species, particularly when small input amounts are used.

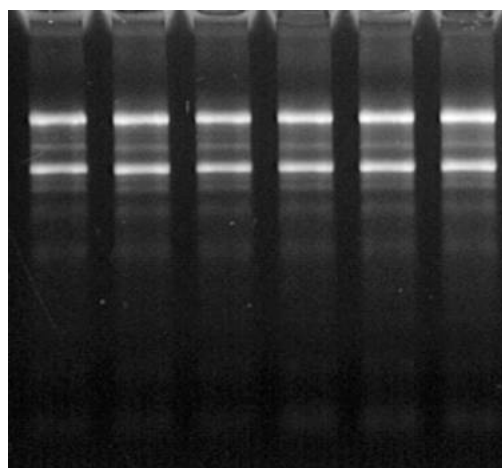


Figure 2. Consistent Isolation of Leukocyte RNA. Total RNA was isolated from 200 μ L of whole hamster blood using Norgen's Leukocyte RNA Purification Kit. A total of six replicates were carried out for the sample preparation procedure. Samples of the purified RNA (5 μ L of the 50 μ L elutions) were loaded onto a 1X MOPS, 1.2% formaldehyde-agarose gel and visualized via ethidium bromide staining. Norgen's kit allowed for the consistent isolation of high quality RNA from all replicates.

In order to assess the biological activity of the purified RNAs, RT-qPCR was performed (**Figure 4**). Total RNA was isolated from 200 μ L of whole hamster blood using Norgen's Leukocyte RNA Purification kit and the leading market competitor's kit. The purified RNA was then used as template in an RT-qPCR reaction for detection of the β -actin gene or the *miR-21* microRNA. **Panel A** shows the successful amplification of the *miR-21* transcript from small RNAs isolated by Norgen's kit. As can be observed, while Norgen's kit was able to isolate the small RNA species, the competitor's kit failed to purify the microRNA as evidenced by the lack of amplification. Norgen's kit isolated similar yields of the large RNA species to the competitor's kit, as indicated by the Ct values when the β -actin gene was amplified (**Panel B**). Therefore, Norgen's Leukocyte RNA Purification kit can isolate not only large RNA but also microRNA, indicating the diversity of RNA species isolated. This further suggests that all species of RNA isolated were of a high purity and had retained their biological activity

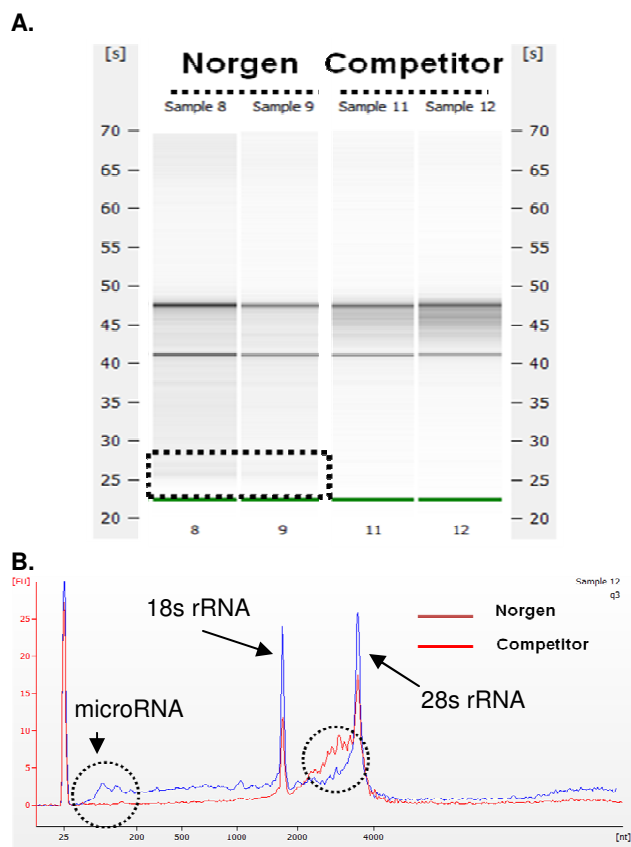


Figure 3. High Quality Leukocyte RNA with Recovery of Small RNAs using Norgen's Kit. Total RNA was isolated from 200 μ L of hamster blood and resolved on an Agilent Lab-On-A-Chip and electropherograms were generated. Panel A contains all the RNA species present in hamster leukocytes isolated with Norgen's Leukocyte RNA Purification kit (in the first two lanes) compared to the RNA species isolated by the leading market competitor's kit. Panel B shows the resultant electropherogram. Norgen's kit purified RNA of a higher yield and quality than the competitor's kit, as indicated by the lack of an additional peak between the two major rRNA bands, a result of RNA degradation. In addition, Norgen's kit recovered all small RNA species, including microRNA.

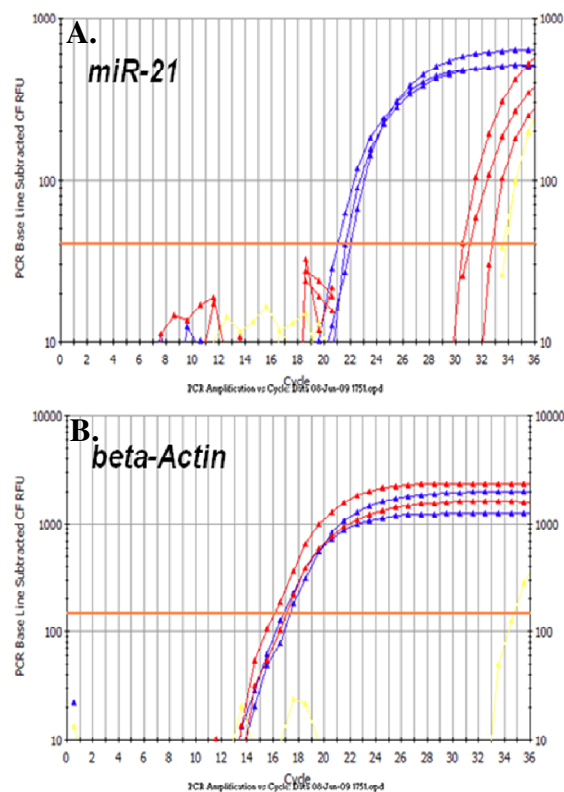


Figure 4. Detection of microRNA from Total Leukocyte RNA Purified using Norgen's Leukocyte RNA Purification Kit. Total RNA was isolated from whole hamster blood and 200 ng was then used to generate cDNA with either *miR-21* stem-loop RT primer or oligo dT. A total of 4 μ L of the generated cDNAs were then used in a 20 μ L qPCR reaction using primers specific for *miR-21* or β -actin. Norgen had the same amplification performance as the leading market competitor for large mRNA (β -actin) (Norgen's sample in blue; leading market competitor's sample in red). However, only Norgen's kit was able to isolate the microRNA, as evidenced by the successful amplification of the *miR-21* gene in Panel A with Norgen's RNA (blue line).

CONCLUSIONS

Norgen's Leukocyte RNA Purification Kit provides a rapid method for the isolation and purification of total leukocyte RNA from mammalian blood samples. Through the analyses of the performance of this kit a number of conclusions regarding the benefits of the kit can be made:

1. Norgen's kit allows for the isolation of high quality total RNA samples within 45 minutes and without the use of any organic solvents. Total RNA, including all small RNA species, are isolated without the use of harmful chemicals such as phenol or chloroform.
2. Selective isolation of leukocyte RNA results in improved expression profiling and other downstream applications by removing the masking effects of some RNAs which are very abundant in whole blood, such as globin mRNAs.
3. The purified RNA is of the highest quality and can be used in a number of downstream applications including:

qPCR, RT-PCR, Northern blotting, RNase protection, primer extension and array analyses requiring the use of intact RNA.

4. Most of the commercial kits currently available that isolate leukocyte RNA do not extract microRNA and special protocols must then be employed to purify the small RNA species.

5. The additional red blood cell lysis step allows the use of higher volumes of blood as input in RNA isolations, resulting in higher yields of leukocyte RNA.

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