Purification of High-quality RNA from Blood Samples with the Thermo Scientific KingFisher Pure RNA Blood Kit

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Technical Note

Goal

This technical note describes the purification of total RNA from up to 250 µL EDTA-treated blood samples, or samples preserved in Tempus or PAXgene tubes, using the Thermo Scientific™ KingFisher™ Pure RNA Blood Kit. The purified total RNA has high integrity and is free of contaminants or inhibitors, providing pure RNA for direct use in various downstream applications.

Introduction

The KingFisher Pure RNA Blood Kit is designed to purify high-quality total RNA from fresh or frozen blood treated with EDTA or citrate, or blood preserved in Tempus or PAXgene tubes. As it is important to take RNA preservation into account throughout purification, the entire process - from sample collection and storage conditions, through to actual RNA purification - should be designed to preserve RNA. These aspects, as well as the donors' white blood cell level, have a great effect on the yield and quality of the purified RNA. Additionally, the RNA purification process should be efficient and fast. When using the KingFisher Pure RNA Blood Kit in combination with the Thermo Scientific™ KingFisher™ Flex or Thermo Scientific™ KingFisher™ Duo magnetic particle processors, purification is rapid and requires very little hands-on time.

Materials and Methods

Total RNA was purified from 200 µL or 250 µL of EDTA-treated blood or PAXgene and Tempus tube-preserved blood samples, using the KingFisher Pure RNA Blood Kit (Cat. No. 98020196) and a KingFisher magnetic particle processor. One run in the KingFisher Duo or KingFisher Flex takes approximately 60 minutes, and after purification the total RNA is eluted into 75 µL of Elution Buffer. This volume can be adjusted.

Eluates from 200 µL of EDTA-treated blood samples were used in cDNA synthesis with the Thermo Scientific™ Maxima™ cDNA Synthesis Kit. Gene expression analysis was performed using the Thermo Scientific™ PikoReal™ 96 instrument together with the Thermo Scientific™ Solaris™ qPCR Gene Expression Master Mix and predesigned Solaris assays targeting GAPDH and PPIH genes.

The performance of the KingFisher Pure RNA Blood Kit in combination with the KingFisher Flex was compared to two different spin column kits. The purification processes were performed in accordance with the instruction manuals of the respective kits, using 250 µL samples for the KingFisher Pure Kit and 500 µL samples for spin column kits.

Results

The average yield and quality of purified RNA using the KingFisher Pure RNA Blood Kit are listed in Table 1. Total RNA was purified from 250 µL of fresh or frozen EDTA blood samples, and from blood preserved in Tempus or PAXgene tubes, using the KingFisher Pure RNA Blood Kit. The samples run on an agarose gel indicate high yield and excellent quality (Figure 1).

Table 1. Typical RNA yields and purities from blood samples*

Blood sample	Typical RNA yield	Purity by absorbance 260/280 nm
200 µL of fresh EDTA treated blood	1.5 - 4 µg	1.9 - 2.0
PAXgene or Tempus stabilized blood	8 - 25 µg	

*RNA yields obtained from blood samples vary individually due to the number of white blood cells.

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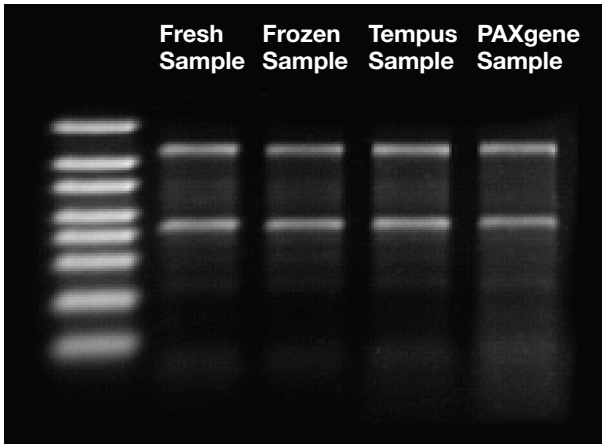

Figure 1. Agarose gel image of total RNA purified from EDTA-treated fresh or frozen blood samples, or samples preserved in Tempus or PAXgene tubes.

For gene expression analysis RNA was purified from 200 µl of fresh blood. After cDNA synthesis the samples were used as templates. The expression analysis indicates that one out of six samples analyzed with PikoReal Software had a 2.5-fold increased expression of the PPIH gene (Figures 2 and 3).

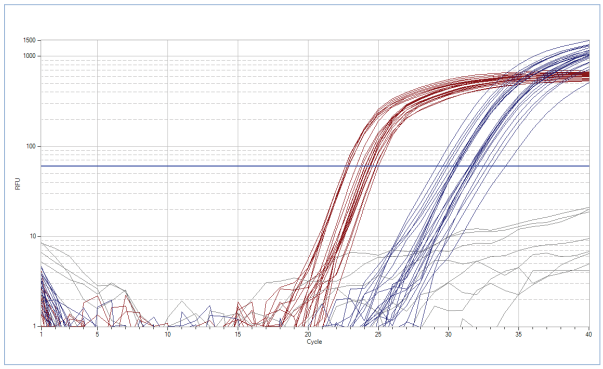

Figure 2. Gene expression amplification graph. Reference gene (GAPDH) in red and target gene in blue (PPIH).

RNA purification was shown to be successful with the KingFisher Pure RNA Blood Kit in combination with the KingFisher Flex, and two spin column kits. However, in comparison with the excellent performance of the KingFisher system, the samples purified with the competitive systems showed lower yields and RNA integrity number (RIN) values (Figure 4).

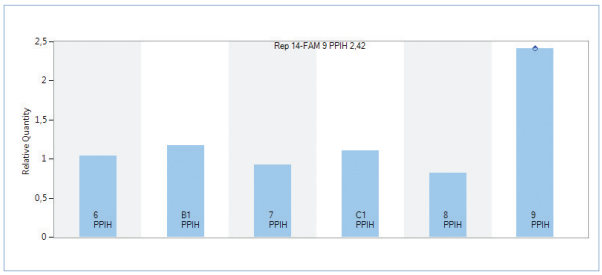

Figure 3. Gene expression showing relative quantity of the target gene (PPIH).

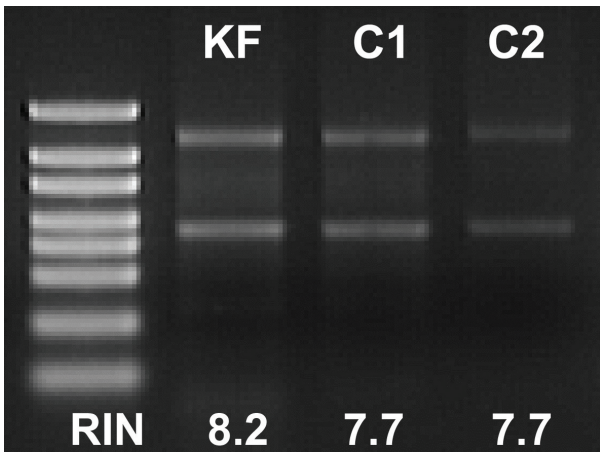

Figure 4. Agarose gel image comparing three different kits: KingFisher Pure RNA Blood Kit (KF) and two different competitor spin column kits (C1 and C2).

Conclusion

The KingFisher Pure RNA Blood Kit efficiently produces purified RNA of high integrity from various types of blood samples. Contaminants or inhibitors are washed away during the process, so the eluate is suitable for direct use in different downstream applications.

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