

Flexible DNA and RNA purification with Thermo Scientific KingFisher Duo and KingFisher Kits

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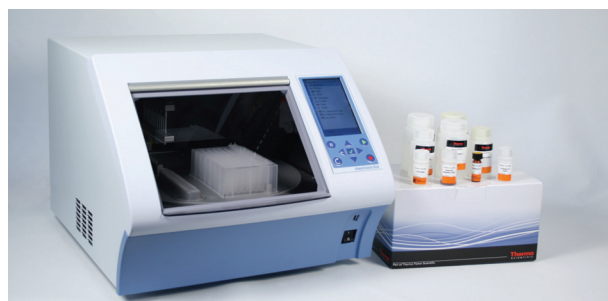
Abstract

Purpose: Evaluation of Thermo Scientific KingFisher Duo magnetic particle processor for DNA/RNA purification by using KingFisher® Nucleic Acid Kits

Methods: Nucleic acid isolation by magnetic separation technology

Results: KingFisher Duo together with KingFisher Kits provide excellent yield and high purity of DNA/RNA from wide variety of sample materials

FIGURE 1. KingFisher Duo and KingFisher Blood DNA Kit



Introduction

New KingFisher Duo magnetic particle processor provides a remarkable alternative for manual nucleic acid isolation for low or medium throughput laboratories. Loss of laborious hands-on time necessary in manual purification protocols with for example spin columns or phenol/chloroform method generate time saving. The magnetic particle technology combines the speed and efficiency of automation with high quality purification capability.

KingFisher Kits accomplish the KingFisher system for DNA and RNA purification from different starting materials, such as blood, cultured cells or bacteria, tissues, cell-free body fluids and plant samples.

The KingFisher technology uses magnetic rods to transfer particles through the various purification phases of binding, mixing, washing and elution, offering a solution with minimized hands-on time.

The new member of the KingFisher instrument family, KingFisher Duo, is easy to adjust for variable processing volumes and sample capacity. The working volume covers range from 20 to 5000 µl and it is possible to process 1-12 samples parallel. High purity and excellent yield of DNA or RNA, free of impurities and contaminants, are achieved by using KingFisher Duo together with KingFisher Kits.

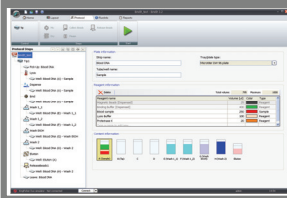
TABLE 1. Maximal sample sizes from different materials for KingFisher Kits

Blood DNA Kit	250 µl or 3 ml blood
Cell and Tissue DNA Kit	1 x 10 ⁷ cells, 20 mg of tissue or 1ml of cultured bacteria
Total RNA Kit	2 x 10 ⁶ cells or 20 mg of tissue
Viral NA Kit	200 µl of cell-free body fluid
Plant DNA Kit	50 mg of fresh plant tissue

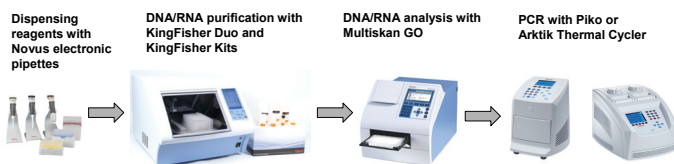
Materials & Methods

The purifications were done using KingFisher DNA and RNA Kits with KingFisher Duo and the BindIt 3.2 software. (Figure 2). The isolation of DNA from different tissues and the competitor comparison from blood samples were performed using KingFisher Flex instrument. The KingFisher Kits used in the experiments were KingFisher Blood DNA Kit, KingFisher Cell and Tissue DNA Kit, KingFisher Viral NA Kit and KingFisher Total RNA Kit. The purity and yield of the DNA and RNA were analyzed with Thermo Scientific Multiskan GO. PCR products were run on an Agilent Bioanalyzer 2100.

FIGURE 2. Example of a protocol for KingFisher Kit with BindIt software 3.2



DNA/RNA isolation and analysis workflow

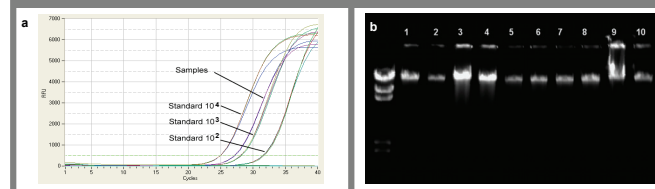


Results

KingFisher Cell and Tissue DNA Kit

DNA from human buccal swabs and three different mouse tissues was purified with KingFisher Cell and Tissue DNA Kit. Buccal swabs and mouse ear, liver or kidney were lysed in the Lysis Buffer including Proteinase K for one hour and the purification procedure was performed. The results indicate good yield of DNA (Figure 3).

FIGURE 3. a. DNA was purified from buccal swabs with KingFisher Duo and qPCR was performed with PikoReal. b. After lysing and purification of DNA with KingFisher Flex mouse samples were run on an agarose gel. The samples in the wells: 1-2 ear samples, 3-4 liver 15 mg, 5-6 liver 10 mg, 7-8 kidney 15 mg, 9-10 kidney 10 mg



KingFisher Blood DNA Kit

KingFisher Blood DNA Kit was used for DNA purification from 250 µl of EDTA treated blood (Figure 4a). In addition, the KingFisher Kit was compared to three competitive methods or instruments (Figure 4b). In the competitor comparison DNA was purified from 200-250 µl blood according to instruction manuals. The results indicate that the performance of KingFisher Blood DNA Kit was excellent.

FIGURE 4.a. DNA purified from EDTA treated blood with KingFisher Duo. b. Highest yield of genomic DNA was isolated by using KingFisher Blood DNA Kit and KingFisher Flex (S1 = spin column, Ca = competitor magnetic particle kit, KF = KingFisher, Cb = competitor purification automate)

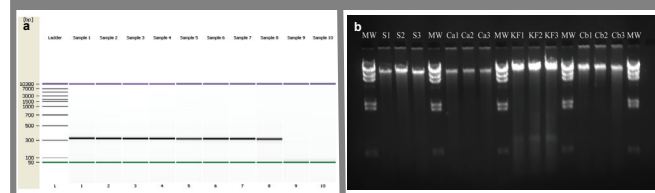
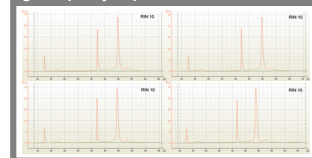


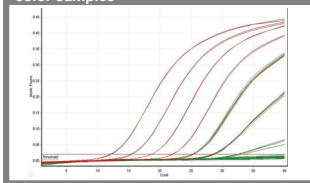
FIGURE 5. Electropherograms of four different RNA samples purified with KingFisher Duo show consistency and good quality of purified RNA



KingFisher Total RNA Kit

KingFisher Total RNA Kit was used for purification of RNA from 5 x 10⁶ HeLa-S3 cells and the quality of the purified RNA was analyzed with Agilent Bioanalyzer 2100. The results show consistency and high quality of RNA (Figure 5). The RNA integrity number (RIN) of the samples was 10, indicating that RNA was intact in all of the samples.

FIGURE 6. Purified RNA was used for qPCR to analyze the quantity of the HBV in the serum samples. The red color indicates standard curves and the green color samples



KingFisher Viral NA Kit

KingFisher Viral NA Kit has excellent sensitivity for the purification of viral NA, which is often scarce in the sample. Viral NA of HBV was purified from serum samples and the quality of the samples was analyzed with qPCR (Figure 6). The samples showed excellent performance in the qPCR.

Conclusions

- KingFisher Duo provides processing of 1-12 samples for up to 5 ml
- Possibility to run two protocols sequentially, up to 24 samples
- Walk-away solution for small and medium size laboratories
- Flexible KingFisher system with optimized purification kits
- Customized kits for a wide variety of sample types
- Proven excellent performance and reproducibility

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