High-throughput purification of DNA and RNA with Thermo Scientific KingFisher Kits

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Abstract

Purpose: Evaluation of Thermo Scientific KingFisher Kits for DNA/RNA purification
Methods: Thermo Scientific KingFisher magnetic particle purification system
Results: KingFisher® Kits provide excellent yield and high purity of DNA/RNA from all tested sample materials

Introduction

New KingFisher Kits accomplish the KingFisher magnetic particle 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 flexible throughput 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. High purity and excellent yield of DNA or RNA, free of impurities and contaminants, are achieved by using KingFisher Kits.

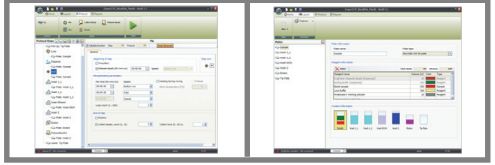
TABLE 1. The table indicates maximal sample sizes from different materials for five KingFisher Kits

Blood DNA Kit	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

Following sample materials were used: blood, mouse tissues, HeLa-S3 cells, fresh tobacco leaves and serum. All five KingFisher Kits were analyzed (Table 1). The purifications were done with Thermo Scientific KingFisher Flex and BindIt software 3.1 (Figure 1). The liquid handling was performed by using either Thermo Scientific Multidrop Combi or Versette. 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 1. Example of a protocol for KingFisher Kit with BindIt software 3.1



Results

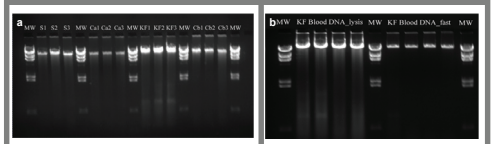
KingFisher Blood DNA Kit

KingFisher Blood DNA Kit was compared to three competitive method or instrument. 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 2a).

Two different BindIt protocols of big volumes were tested by using KingFisher Flex 24-well format with 3 ml of blood. The walk-away protocol including lysis within instrument was compared to the original protocol in the kit insert. The walk-away protocol resulted good quality DNA for secondary applications though yields were lower, as was expected (Figure 2b).

FIGURE 2.a. Highest yield of genomic DNA was isolated by using KingFisher Blood DNA Kit (S1 = spin column, Ca = competitor magnetic particle kit, KF = KingFisher, Cb = competitor purification automate)

b. DNA purified from 3 ml of blood by lysing the sample outside (KF Blood DNA_lysis) and inside (KF Blood DNA_fast) the KingFisher Flex

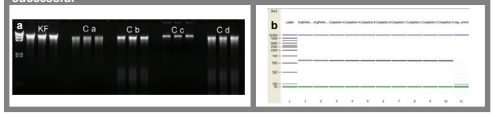


KingFisher Plant DNA Kit

KingFisher Plant DNA Kit was compared to kits from four different competitors. DNA was purified from 50 mg of fresh tobacco leaves. The results indicate that the performance of KingFisher Plant DNA Kit was excellent (Figure 3).

FIGURE 3.a. Agarose gel shows that with the KingFisher Plant DNA Kit (KF) higher yield of DNA was obtained compared to the kits from four different competitors (C a-d)

b. PCR was performed from the purified samples and all the samples were successful

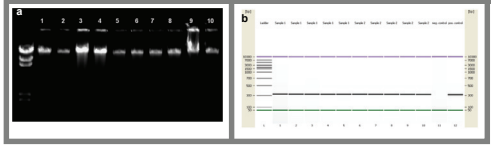


KingFisher Cell and Tissue DNA Kit

DNA from three different mouse tissues was purified with KingFisher Cell and Tissue DNA Kit. Mouse ear, liver or kidney were lysed in the Lysis Buffer including Proteinase K for one hour and the purification procedure was performed in the KingFisher Flex. The results indicate good yield of DNA (Figure 4a).
DNA was purified from 50 000 and 100 000 of HeLa-S3 cells and the samples were used for PCR. The PCR products run on an Agilent Bioanalyzer 2100 showed that the PCR was successful from all of the samples (Figure 4b).

FIGURE 4.a. DNA purified from mouse tissue samples was run on an agarose gel. The samples: 1-2 ear samples, 3-4 liver 15 mg, 5-6 liver 10 mg, 7-8 kidney 15 mg, 9-10 kidney 10 mg

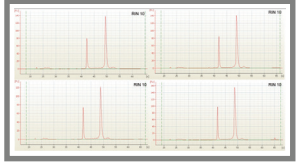
b. DNA was purified from 50 000 (sample1, five parallel samples) or 100 000 (sample 2, five parallel samples) HeLa-S3 cells and the PCR was performed using primers amplifying 330 bp long DNA strand



KingFisher Total RNA Kit

KingFisher Total RNA Kit was used for purification of RNA from 1 x 10^6 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.

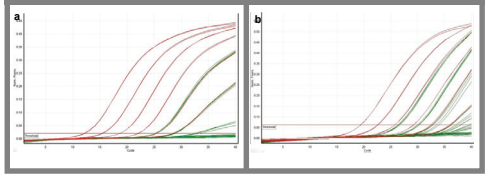
FIGURE 5. Electropherograms of four different RNA samples show consistency and good quality of purified RNA



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 and HCV were purified from serum samples and the quality of the samples was analyzed with qPCR (Figure 6). The samples showed excellent performance in the qPCR.

FIGURE 6. Purified RNA was used for qPCR to analyze the quantity of the HBV (a) and HCV (b) virus in the serum samples. The red color indicates standard curves and the green color shows different samples



Conclusions

- Flexible KingFisher system with optimized purification kits
- Customized kits for a wide variety of sample types
- Proven excellent performance and reproducibility
- Walk-away solution for any throughput

DNA/RNA isolation and analysis workflow

Liquid handling with Multidrop Combi and Versette

DNA/RNA purification with KingFisher Flex and KingFisher Kits

DNA/RNA analysis with Multiskan GO

PCR with Piko Thermal Cycler

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