Forget Tedious Manual Purification – Try Thermo Scientific KingFisher Duo

SP&A Application Laboratory, Thermo Fisher Scientific, Vantaa, Finland

Goal

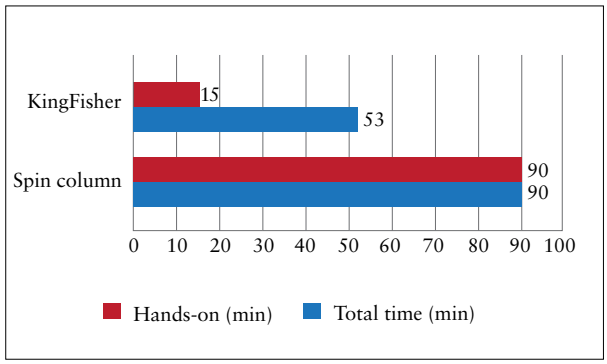
Thermo Scientific™ KingFisher™ Duo, a magnetic particle processor, is an automated system for the extraction of nucleic acids, proteins and cells. It enhances the purification process and shortens tedious hands-on time, yet it has a small footprint and is easy to use. In this technical note we show that nucleic acids purified with the KingFisher Duo have higher yield and quality with shorter time used for the process when compared to spin column-based manual methods.

Introduction

Extraction of small molecules, such as nucleic acids and proteins, is an increasingly popular application in life science research. The KingFisher Duo is a magnetic particle processor, extracting and purifying selected small molecules bound to magnetic particles.

Automated purification with the KingFisher Duo gives the user an opportunity to remarkably shorten the hands-on time spent for the extraction. For example, widely used spin column-based methods require constant manual handling of the samples throughout the purification process. In contrast to laborious manual steps, extraction with the KingFisher Duo requires only preparation of the plate and loading it to the system.

The KingFisher Duo is suitable for low- and medium-throughput laboratories, processing 12 samples, or even 24 from one loading, in up to 1 ml processing volume. The instrument includes also large volume processing up to 5 ml with six samples per run. These options give flexibility to the sample size and number of samples processed. The typical run time per protocol is 30–45 minutes, so within one day it is possible to process more than 100 samples. In case of nucleic acids, the eluates are often required to be of uniform quality and suitable for enzymatic downstream applications. With the KingFisher Duo these requirements can easily be covered.



	Hands-on time	Total time
KingFisher Duo	15 min	53 min
Spin column	90 min	90 min

Table 1. Time comparison of the KingFisher Duo and spin columns shows the fast and effortless purification with the KingFisher Duo. gDNA was purified from 200 µl of blood samples.

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Materials and methods

Nucleic acid purifications were performed to compare the KingFisher Duo to a spin column kit and two magnetic particle-based systems from other manufacturers. The purifications were performed from 200 µl of fresh EDTA treated blood and 1 x 10^6 HeLa-S3 cells. Thermo Scientific KingFisher Pure DNA Blood Kit (cat. no. 98010196) or KingFisher Pure RNA Blood Kit (cat. no. 98020196) was used together with the KingFisher Duo for DNA and RNA purification from blood samples. Purification from the cultured HeLa-S3 cells was performed with the Thermo Scientific KingFisher Cell and Tissue DNA Kit (cat. no. 97030196) and KingFisher Pure RNA Tissue Kit (cat. no. 98040196). In the case of the spin column kit and magnetic particle systems from other vendors, the purifications were performed in accordance with the instruction manuals of the respective kits. The time used for the purification with the KingFisher Duo or spin columns was followed. In preparation of the KingFisher Duo the buffers were transferred to the empty wells with the stepper function in Thermo Scientific Finnpipette Novus single channel pipette (cat. no. 46200600 and 46200700).

Results were analyzed with the Thermo Scientific Multiskan GO (cat. no. 51119300), Agilent 2100 Bioanalyzer (Agilent Technologies) and RiboGreen assays on Thermo Scientific Fluoroskan Ascent (cat. no. 5210470).

Results

Purification with the KingFisher Duo saves time

The extraction of gDNA from 12 blood samples on the KingFisher Duo took 53 minutes, from which the hands-on time was 15 minutes (Table 1). Correspondingly, the extraction of 12 samples with the spin columns took 90 minutes and required constant attendance of the user. Similar time difference in favorite with the KingFisher Duo and KingFisher Pure RNA Tissue Kit was seen in purification of RNA from HeLa-S3 cell samples, as well as RNA purification from blood samples with the KingFisher Pure RNA Blood kit (data not shown). To accelerate the purification process the Finnpipette Novus pipette stepper function was used in preparation of the plate. Using the stepper in pipetting during the spin column purification is not possible due to increased risk of cross-contamination caused by transferring the same tip between different samples.

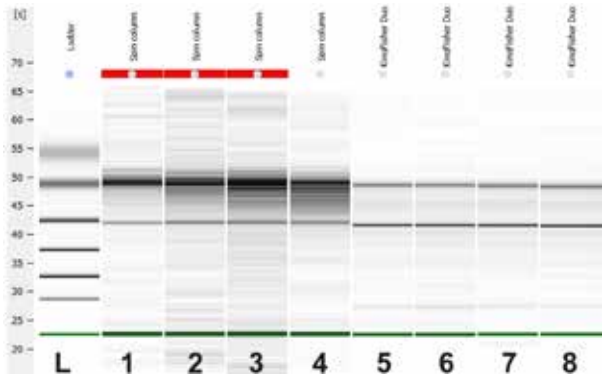

Figure 1. The RNA purified from 200 µl of blood with the KingFisher Duo or spin column kit were run on Agilent Bioanalyzer. The results indicate the purification with the KingFisher Duo gave the highest quality of RNA. Lanes: L ladder; 1-4 spin column kit; 5-8 KingFisher Duo.

Highest RNA yield and quality with the KingFisher Duo

Intact RNA purification from blood is a challenging application. The yield and quality of RNA purified with the KingFisher Duo was compared to the RNA purified with corresponding spin column kit. The eluates were run in the Agilent Bioanalyzer. The RIN (RNA integrity number) value was 8.1 in the samples purified with the KingFisher Duo but with the spin column kit the values were remarkable lower, indicating low yield and degraded RNA (Figure 1). RIN value, varying from 1 to 10, is used to estimate the integrity of total RNA. The RIN 8.1 indicates excellent quality. Additionally, the fluorometric and spectrophotometric analyses indicated higher RNA yield was purified with the KingFisher Duo compared to the spin column kit (Table 2).

	Sample volume	RNA yield F	RNA yield S	A_{260}/A_{280}
KingFisher Duo	200 µl	1.82 µg	2.17 µg	1.9
Spin column	200 µl	0.42 µg	0.36 µg	1.9

Table 2. The total RNA yield purified from 200 µl of blood with the KingFisher Duo gave highest yield of RNA compared to the spin columns according to the fluorometric (F) and spectrophotometric (S) analyses.

Highest gDNA yield purified with the KingFisher Duo

DNA was purified from 1 x 10^6 HeLa-S3 cells with the KingFisher Duo and KingFisher Cell and Tissue DNA Kit, spin column kit or two magnetic particle systems from other vendors. The agarose gel picture indicates that with the KingFisher Duo it was possible to purify highest yield of high-quality DNA (Figure 2, table 3).

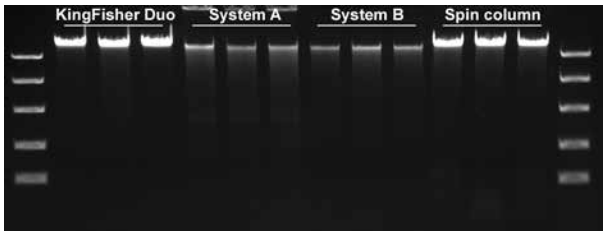

Figure 2. DNA was purified from 1 x 10^6 HeLa-S3 cells with the KingFisher Duo, spin columns and two magnetic particle systems from other manufacturers.

	KingFisher Duo	System A	System B	Spin column
Total gDNA yield (µg)	20.6	10.2	5.3	15.3
A_{260}/A_{280}	1.96	1.92	2.08	1.97

Table 3. KingFisher Duo produced highest yield of DNA compared to two magnetic bead systems (system A and B) and a spin column kit.

Conclusion

Automation of the nucleic acid extraction process has many benefits compared to the manual methods with spin columns. Purification with the KingFisher Duo results in consistent and reproducible DNA and RNA of high yield and quality. The KingFisher Duo saves time and laborious hands-on time can be remarkably shortened in comparison to spin column-based methods. The instrument is easy to use – preparing and starting the run is straightforward, and there are kits and protocols available for various applications. The applications include nucleic acid extraction from different sample materials, but also several proteomic applications and cell separation. Besides being a user-friendly and easy-to-use system, the KingFisher Duo has a small footprint and moderate operating costs.

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North America: +1 800 522 7763
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Belgium +32 2 482 30 30
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France +33 2 28 03 20 00
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Germany International +49 6184 90 6940
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