

Purification of High-quality RNA from Cell and Tissue Samples with the Thermo Scientific KingFisher Pure RNA Tissue Kit

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Goal

This technical note describes total RNA purification from several mammalian cell lines, various tissue samples, and microbial cultures with the Thermo Scientific™ KingFisher™ Pure RNA Tissue Kit. The purified total RNA has high integrity and is free of contaminants or inhibitors, thus providing pure RNA for direct use in various downstream applications.

Introduction

The KingFisher Pure RNA Tissue Kit is designed to purify high-quality total RNA from mammalian cells, tissues, yeasts, and bacteria. As it is important to pay close attention to RNA preservation during purification, the entire process - from sample collection and storage conditions, through to actual RNA purification - should be designed to preserve RNA. These aspects, together with the sample material and homogenization process, have a considerable effect on the yield and quality of the purified RNA.

The efficiency and speed of the RNA purification process are also extremely important, and with the KingFisher Pure RNA Tissue Kit in combination with the Thermo Scientific™ KingFisher™ Flex or Thermo Scientific™ KingFisher™ Duo magnetic particle processors, purification is both rapid and requires very little hands-on time. The KingFisher Flex instrument enables automation of 96 samples in a high-throughput format, while a lower throughput instrument, the KingFisher Duo, is available for up to 12 samples per run.

Materials and Methods

Total RNA was purified from three mammalian cell lines, seven mouse tissues, one bacterial, and one yeast culture using the KingFisher Pure RNA Tissue Kit (Cat. No. 98040196) in combination with KingFisher magnetic particle processors. The sample types and amounts used for the purification are specified in Table 1. Additional reagents were necessary for lysis of the yeast and bacterial samples. One run on the KingFisher Duo or KingFisher Flex takes approximately 60 minutes, and after purification, the total RNA was eluted into 100 µL of nuclease-free water. The elution volume can be adjusted with the Thermo Scientific™ BindIt™ software.

The reproducibility of RNA purification using the KingFisher Pure RNA Tissue Kit was verified by purifying total RNA from 2×10^6 cultured HeLa cells using the KingFisher Flex and KingFisher Duo.



Results

Total RNA eluates from the 12 different sample types were analyzed by running them on 1% agarose gel and then on the Agilent Bioanalyzer RNA 6000 Nano Chip. Two clear bands on the gel and high RNA integrity number (RIN) values indicate the high yield and excellent quality of the RNA (Figure 1, Table 1).

Table 1. RIN values from total RNA purified from various sample materials with the KingFisher Pure RNA Tissue Kit

No.	Sample material	Starting amount	RIN
1	Jurkat cells	1×10^6 cells	8.1
2	HeLa cells	1×10^6 cells	9.8
3	Cos-7 cells	5×10^5 cells	10
4	Mouse heart	5 mg	8.9
5	Mouse liver	5 mg	7.8
6	Mouse brain	5 mg	8.4
7	Mouse lung	5 mg	7.2
8	Mouse muscle	5 mg	7.2
9	Mouse kidney	5 mg	8.5
10	Mouse spleen	5 mg	9.2
11	<i>E. coli</i> ER2267	1 mL overnight culture	9.4
12	<i>Saccharomyces cerevisiae</i>	1 mL overnight culture	N/A

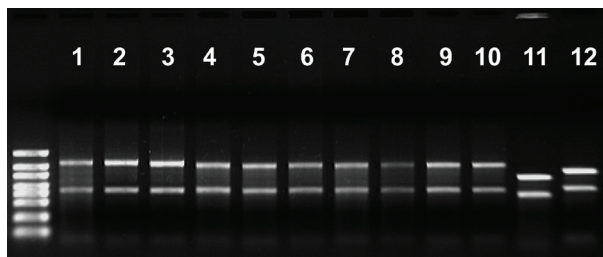


Figure 1. Agarose gel image of total RNA purified from various sample materials listed in Table 1.

The total RNA yield from various sample materials was calculated based on the absorbance measurements at 260 nm (Figure 2). The purification of total RNA from cultured HeLa cells was shown to be consistent and the RIN numbers in Figure 3 indicate excellent reproducibility of the KingFisher system from well to well.

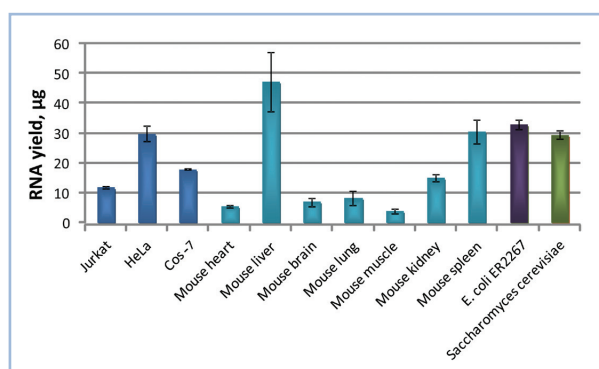


Figure 2. The total RNA yield (µg) from various sample materials, three replicates of each sample.

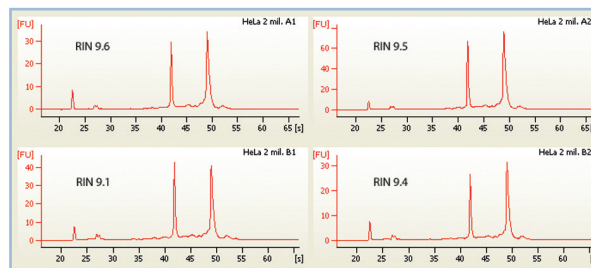


Figure 3. The RNA integrity analysis of total RNA purified from HeLa cells using the KingFisher Pure RNA Tissue Kit shows consistent results on the Agilent Bioanalyzer 2100.

Conclusion

The KingFisher Pure RNA Tissue Kit efficiently produces purified RNA of high integrity from various tissue 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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