Isolation of total RNA with the Amersham RNAspin Midi Isolation Kit

Intellectual Property Notice: The Biopharma business of GE Healthcare was acquired by Danaher on 31 March 2020 and now operates under the Cytiva™ brand. Certain collateral materials (such as application notes, scientific posters, and white papers) were created prior to the Danaher acquisition and contain various GE owned trademarks and font designs. In order to maintain the familiarity of those materials for long-serving customers and to preserve the integrity of those scientific documents, those GE owned trademarks and font designs remain in place, it being specifically acknowledged by Danaher and the Cytiva business that GE owns such GE trademarks and font designs.

cytiva.com

CY14240-08Jun20-AN

Application note 28-4070-86 AA RNA isolation

Isolation of total RNA with the illustra RNAspin Midi Isolation Kit

Key words: *illustra ● total RNA ● microarray ● quantitative reverse transcription polymerase chain reaction (QRT-PCR) ● primer extension ● Northern blot ● sample preparation ● high-throughput*

The illustra™ RNAspin Midi Isolation Kit can be used to isolate total RNA from cultured cells, tissue, bacteria and yeast. The procedure is straightforward and takes less than 30 min to complete. The isolated RNA is of sufficient quantity and quality for downstream applications, including Northern blot analysis, quantitative reverse transcription polymerase chain reaction (QRT-PCR), primer extension or RNase protection assays. The kit is supplied with all the necessary components including prefilters and DNase I. This application note describes the quality and quantity of RNA obtained using the kit and summarizes the results obtained in two downstream applications: quantitative reverse transcription polymerase chain reaction QRT-PCR and cRNA synthesis for a microarray workflow.

Materials

Products used

illustra RNAspin Midi Isolation Kit	28-0500-73
CyScribe™ GFX™ Purification Kit	27-9606-01
CodeLink™ Expression Assay Reagent Kit	320012

Other materials required

Agilent bioanalyzer 2100 (Agilent)

RNA*later*™-ICE buffer (Ambion)

TaqMan™ Reverse Transcription Reagents (Applied Biosystems)

TaqMan TAMRA primer/probe sets (Applied Biosystems)

ABI PRISM 7900HT Sequence Detection System (Applied Biosystems)

Protocol

The RNAspin Midi RNA Isolation Kit was used to purify total RNA for several different applications according to the protocol illustrated in Figure 1. To reduce the risk of clogging the RNA-binding column and the potential loss in sample yield and purity, the lysates in these experiments were prefiltered using column filters included in the kit. Incubation with DNase I, also included in the kit, was efficiently carried out on-column in conditions optimized by the Membrane Desalting Buffer.

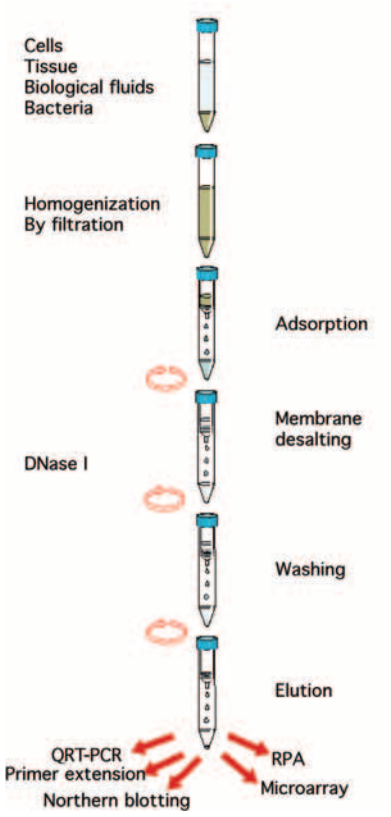

Fig 1. Schematic representation of the RNAspin Midi Isolation Kit protocol.

Assessment of RNA quality

The RNAspin Midi Kit was used to isolate total RNA from five separate 100-mg aliquots of rat live tissue and four independent 100-mg aliquots of human spleen tissue. One microliter of the total 500-µl eluate was evaluated on the Agilent 2100 bioanalyzer.

Application of the total RNA product in quantitative RT-PCR

A 100-mg aliquot of rat liver tissue was frozen and subsequently stored in RNA*later*-ICE buffer. Total RNA was extracted using the RNAspin Midi RNA Isolation Kit and cDNA was generated using TaqMan Reverse Transcription Reagents and TaqMan TAMRA primer/probe sets with 1 µg of the RNA templates (1). The probe/primer set targeted the rat thyroid hormone sulfotransferase *(ST1B1)* gene. *GAPDH* was used as a control transcript. The reactions were then purified using the CyScribe GFX Purification Kit. Different amounts (4.0 ng, 0.4 ng, and 0.04 ng) of the purified cDNA were used for QRT-PCR with the HotStarTaq™ Kit (Qiagen) on the ABI PRISM 7900HT Sequence Detection System.

Application of the total RNA product in labeled-cRNA synthesis for a microarray workflow

The RNAspin Midi Isolation Kit was used to purify total RNA from five independent rat liver tissue samples. After isolation in parallel, 1 µg of each of the total RNA products was used to generate separate preparations of biotin-labeled cRNA using the CodeLink Expression Assay Reagent Kit. The purity and integrity of the labeled cRNA was evaluated by A_{260}/A_{280} spectrophotometry and on the Agilent 2100 bioanalyzer.

Results

Assessment of RNA quality

An important aspect of total RNA purity is whether or not it is contaminated with genomic DNA post-isolation. The membrane-desalting step of the RNAspin Midi kit creates an optimal chemical environment for efficient on-column DNase I digest. Thus, the isolated total RNA is free of genomic DNA (gDNA). This is especially critical for downstream applications such as cDNA synthesis that involve enzymatic reactions in which contaminating gDNA may interfere and cause spurious product formation and/or low product yield. Figure 2 describes the results of isolating total RNA from four independent human spleen samples (Fig 2A) and five independent rat liver samples (Fig 2B) using the RNAspin Midi kit. The A_{260}/A_{280} ratios were all between 1.9 and 2.2. In addition to the high RNA purity, high RNA integrity was obtained, with RIN values typically greater than 7 (Fig 2A and 2B). The RNAspin Midi kit produced high-quality product (Fig 2A) even with tissues (such as human spleen) that have proven difficult to process with traditional RNA isolation methods. Traditional Northern blot analysis requires greater amounts of total RNA (up to 10 µg per lane (1). The RNAspin Midi kit routinely produces sufficient amounts of RNA for this application. In Figure 2C, we highlight the typical yields of about 700 µg total RNA from 100 mg of rat liver input material.

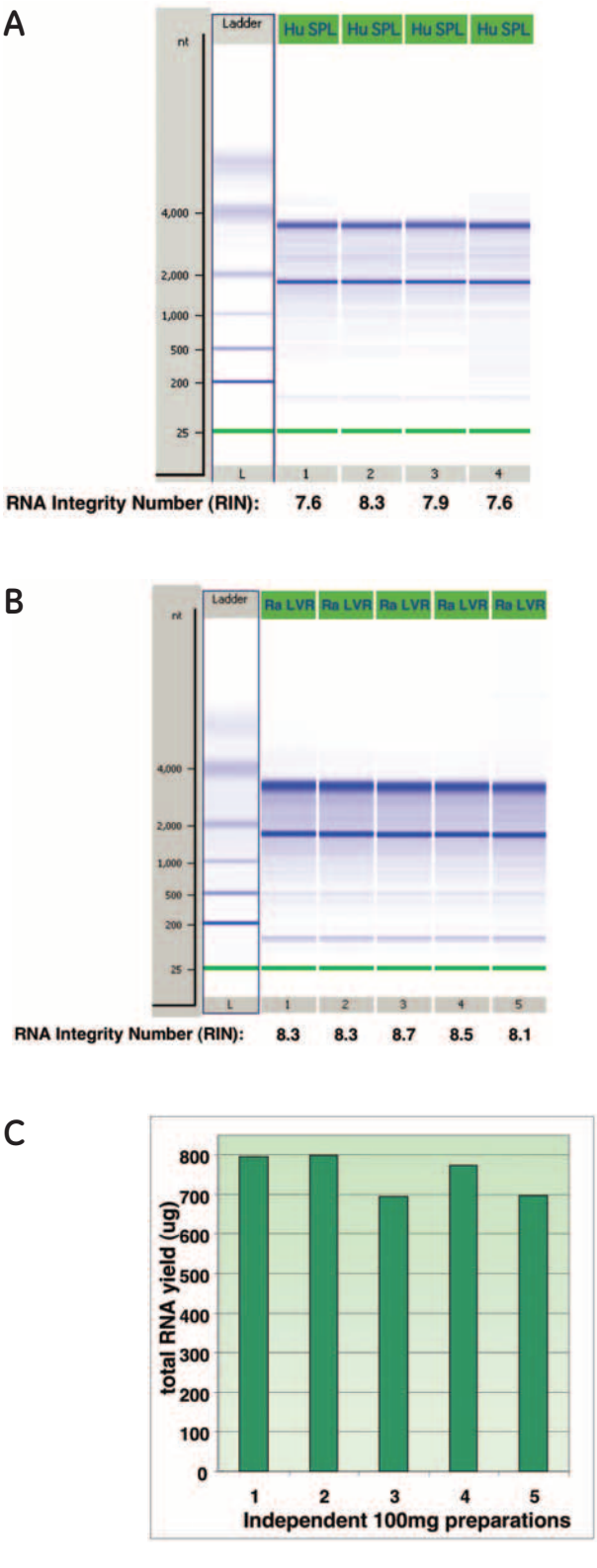

Fig 2. Total RNA from five independent tissue aliquots was isolated with the RNAspin Midi kit. (A) Quality of RNA from human spleen tissue; (B) Rat liver tissue on the Agilent 2100 bioanalyzer; (C) Total RNA yields calculated from A_{260} spectrophotometer readings for the rat liver samples.

Application of the total RNA product in QRT-PCR

The rat thyroid hormone, sulfotransferase, which is encoded by the *ST1B1* gene, plays an important role in the regulation of thyroid hormone metabolism. As with other important transcripts, measurement of the relative expression level in the liver is vital to the understanding of the biological function of the hormone. The data presented in Figure 3 demonstrates that the RNAspin Midi kit is capable of isolating high-quality RNA for sensitive downstream applications like QRT-PCR (Fig 3).

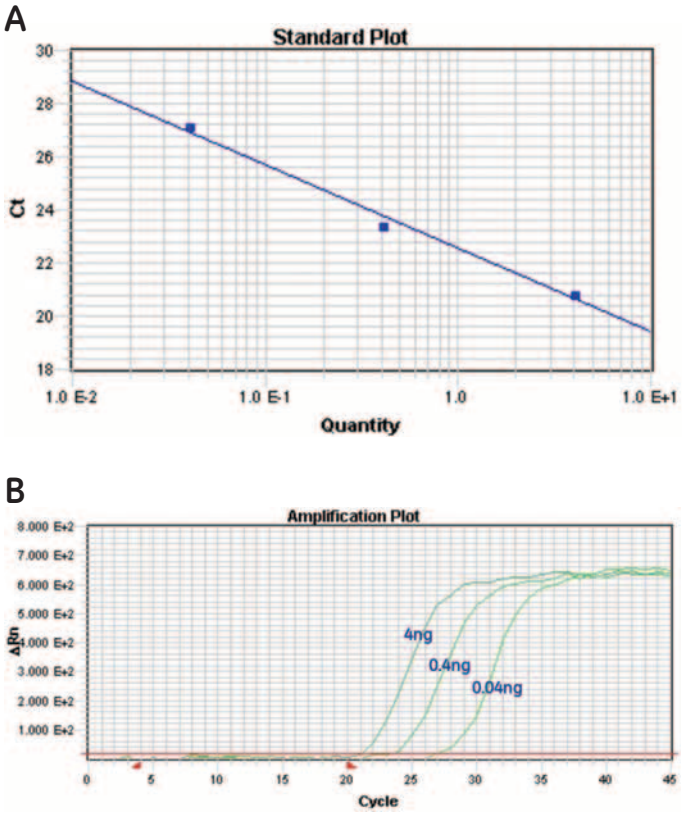

Fig 3. The RNAspin Midi Kit total RNA product performs well in sensitive downstream applications such as QRT-PCR. Titration of an RNAspin Midi total RNA sample (NAP287) with TAQ100 primer/probe set (rat thyroid hormone sulfotransferase, *ST1B1* gene). (A) QRT-PCR standard curve indicating the appropriate 10-fold differences in the reaction dilutions; (B) QRT-PCT CT curves showing typical response curves for a clean RNA template.

Evaluation of total RNA efficacy in cDNA synthesis and subsequent labeled-cRNA amplification for microarray work

The average A_{260}/A_{280} ratio of the cRNA generated from rat liver total RNA product using the RNAspin Midi kit was 2.19. Analysis of the cRNA on an Agilent 2100 bioanalyzer (Fig 4) showed that the labeled cRNA was of consistent high yield and integrity. The total RNA input material successfully generated labeled-cRNA in a reproducible manner thus demonstrating that biologically relevant microarray results can be produced from RNAspin Midi kit RNA.

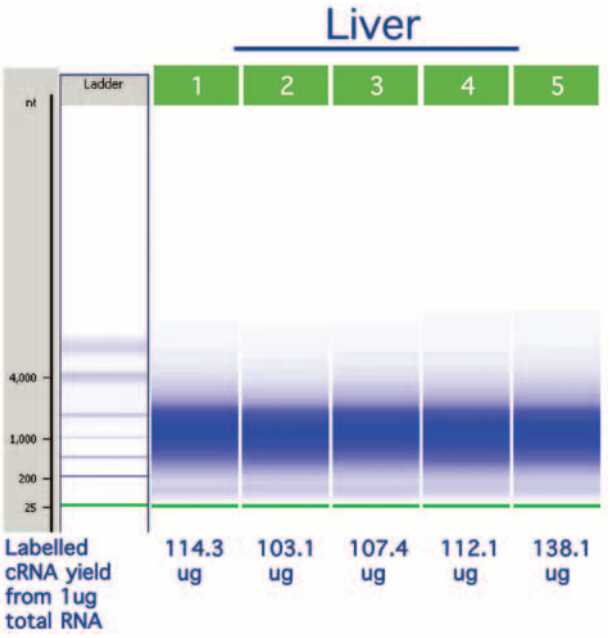

Fig 4. Analysis of labeled-cRNA quality on the Agilent bioanalyzer 2100 demonstrates reproducible cRNA quality and yield.

Conclusions

The RNAspin Midi RNA Isolation kit yields RNA of high quality and integrity with little or no contaminating gDNA. The RNA product can be purified in varying amounts from a diverse range of sample types, including samples that are difficult to process. The quality, integrity, and yield of total RNA is high enough for the most sensitive of downstream applications such as QRT-PCR, Northern blotting and microarray analysis.

Reference

1. Ausubel, F.A., ed., Short protocols in molecular biology: a compendium of current protocols in molecular biology, John Wiley and Sons, Inc. Indianapolis, IN (2002).

Asia Pacific	Tel: +85 65 62751830	Fax: +85 65 62751829
Australasia	Tel: +61 2 8820 8299	Fax: +61 2 8820 8200
Austria	Tel: 01 /57606 1613	Fax: 01 /57606 1614
Belgium	Tel: 0800 73 890	Fax: 02 416 8206
Canada	Tel: 1 800 463 5800	Fax: 1 800 567 1008
Central & East Europe	Tel: +43 1 972 720	Fax: +43 1 972 722 750
Denmark	Tel: +45 70 25 24 50	Fax: +45 45 16 2424
Eire	Tel: 1 800 709992	Fax: +44 1494 542010
Finland & Baltics	Tel: +358 9 512 3940	Fax: +358 9 512 39439
France	Tel: 01 69 35 67 00	Fax: 01 69 41 98 77
Germany	Tel: 0800 9080 711	Fax: 0800 9080 712
Greater China	Tel: +852 2100 6300	Fax: +852 2100 6338
Italy	Tel: 02 26001 320	Fax: 02 26001 399
Japan	Tel: 81 3 5331 9336	Fax: 81 3 5331 9370
Korea	Tel: 82 2 6201 3700	Fax: 82 2 6201 3803
Latin America	Tel: +55 11 3933 7300	Fax: +55 11 3933 7304
Middle East & Africa	Tel: +30 210 96 00 687	Fax: +30 210 96 00 693
Netherlands	Tel: 0800-82 82 82 1	Fax: 0800-82 82 82 4
Norway	Tel: +47 815 65 777	Fax: +47 815 65 666
Portugal	Tel: 21 417 7035	Fax: 21 417 3184
Russia, CIS & NIS	Tel: +7 495 956 5177	Fax: +7 495 956 5176
Spain	Tel: 902 11 72 65	Fax: 935 94 49 65
Sweden	Tel: 018 612 1900	Fax: 018 612 1910
Switzerland	Tel: 0848 8028 10	Fax: 0848 8028 11
UK	Tel: 0800 515 313	Fax: 0800 616 927
USA	Tel: +1 800 526 3593	Fax: +1 877 295 8102

www.gehealthcare.com/nap

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

imagination at work

Application note 28-4070-86 AA 2006-05