



# Isolation of total RNA with the Amersham RNAspin 96 Isolation Kit

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# Isolation of total RNA with the illustra RNAspin 96 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 96 Isolation Kit can be used to reproducibly (well-to-well, plate-to-plate) isolate total RNA from cultured cells, tissue, bacteria or yeast. It allows for the simultaneous processing of 96 samples via either centrifugation or vacuum-based protocols that can easily fit onto existing automated systems. The isolated RNA is of a high enough quality and quantity (up to 100 µg) for multiple downstream experiments, including sensitive enzymatic applications like quantitative reverse transcription polymerase chain reaction (QRT-PCR), primer extension and RNase protection assays. Thus, the RNAspin 96 kit is a flexible, high-throughput solution for total RNA isolation. This application note describes results obtained from using both the manual centrifugation as well as an automated vacuum workflow to isolate total RNA from several types of source material and their subsequent use in QRT-PCR.

## Materials

### Products used

illustra RNAspin 96 Isolation Kit	28-0500-74
RNAspin 96 Filter Plate	25-0500-88
CyScribe™ GFX™ Purification Kit	RPN5660X
Hybond™N Membrane	RPN82N

### Other materials required

Agilent 2100 bioanalyzer (Agilent)  
 Tecan Genesis 150 Liquid Handling System  
 (Tecan Trading AG)

RNAlater™-ICE buffer (Ambion)

SYBR™ Green (Molecular Probes)

MultiProbe™ II HT Liquid Handling System (Perkin Elmer)

Biomek™ FX Liquid Handling System (Beckman Coulter)

ABI 7900 instrument (Applied Biosystems)

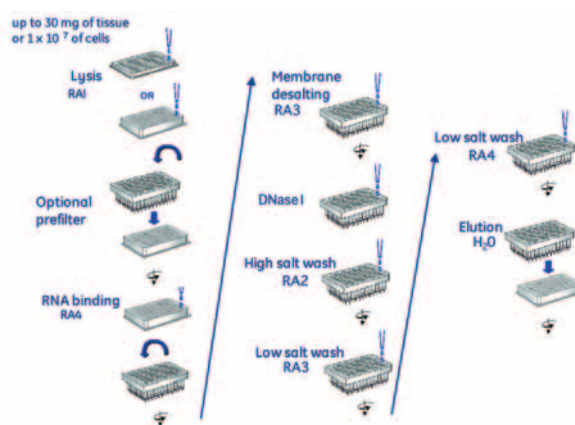
TaqMan™ Reverse Transcription Reagents (Applied Biosystems)

TaqMan TAMRA primer/probe sets (Applied Biosystems)

LightCycler™ Detection System (Roche Applied Science)

## Protocol

The RNAspin 96 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 plate wells and the potential loss in sample yield and purity, the lysates were prefiltered using the optional RNA Filter Plate. Incubation with DNase I—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 96 kit centrifugation protocol



## Isolation of total RNA

Experiment 1: Frozen rat liver tissue was stored in RNA/ater-ICE buffer and then aliquoted into several 20-mg samples. Total RNA was extracted from several of these aliquots, over three different 96-well plates, using the centrifugation protocol of the RNASpin 96 kit. Each RNA sample was eluted with 100  $\mu$ l of RNase-free water. A microliter of each eluate from nine of these samples (different wells of the RNASpin 96 preparation over 3 plates) was ran on the Agilent 2100 bioanalyzer.

Experiment 2: HeLa cells ( $5 \times 10^5$ ) from a homogeneous cell culture, grown according to standard procedures (1), were pelleted in each well of a 96-well, cell-culture plate. Total RNA was isolated according to the RNASpin 96 kit as adapted for the Tecan Genesis 150 automated system. Each eluate (20  $\mu$ l from a 100- $\mu$ l elution volume) was subjected to denaturing gel electrophoresis (1). Each sample (15  $\mu$ l) was run on a 1% denaturing gel and subjected to Northern blot analysis using the Hybond-N nylon membrane. *GAPDH* was used as the radioactively labeled probe.

Experiment 3: 48  $\times$  10-mg mouse liver tissue samples were manually processed via the RNASpin 96 kit (vacuum protocol). The tissue was homogenized in RA1 buffer under liquid nitrogen and the lysate was clarified using the RNASpin Filter Plate. Yield and purity were determined using usual spectrophotometric methods (1) for all the 48 samples.

Experiment 4: 30 wells containing 10 mg of mouse liver tissue each were processed for RNA isolation using the RNASpin 96 kit on a Biomek FX vacuum automation system. The RNA was eluted with 80  $\mu$ l of RNase-free water, and 1  $\mu$ l of this was used for QRT-PCR. cDNA was synthesized using *GAPDH* primers and SYBR Green. The level of *GAPDH* transcript was measured with the LightCycler detection system according to the manufacturer's protocol.

### Isolation of total RNA from a relatively large amount of cells ( $1 \times 10^7$ of HeLa cells)

HeLa cells were grown in culture according to standard procedures (1) and diluted to the indicated final cell numbers. Total RNA was isolated from this range of HeLa cells ( $5 \times 10^5$  to  $1 \times 10^7$ ) using the RNASpin 96 kit with the vacuum protocol. Yield and purity were measured by spectrophotometry at  $A_{260}$  and  $A_{280}$ .

### Isolation of total RNA from a relatively small amount of cells ( $\leq 10$ of HeLa cells)

HeLa cells were grown in culture according to standard procedures (1) and diluted to the indicated final cell numbers. Total RNA was extracted from these HeLa cell aliquots using the RNASpin 96 kit with the vacuum protocol

in an automated format on the MultiProbe II HT platform. The cell numbers ranged from  $1-2 \times 10^6$ . The presence of amplifiable RNA was evaluated by RT-PCR using 2  $\mu$ l of the 100- $\mu$ l eluate and *GAPDH* primers.

### 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/ater-ICE buffer. Total RNA was extracted using the RNASpin Mini kit. cDNA was generated using TaqMan Reverse Transcription Reagents and TaqMan TAMRA primer/probe sets with 1  $\mu$ g 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. The purified cDNA (4.0 ng, 0.4 ng, and 0.04 ng) were used for QRT-PCR with the HotStarTaq™ Kit (Qiagen) on the ABI 7900 instrument.

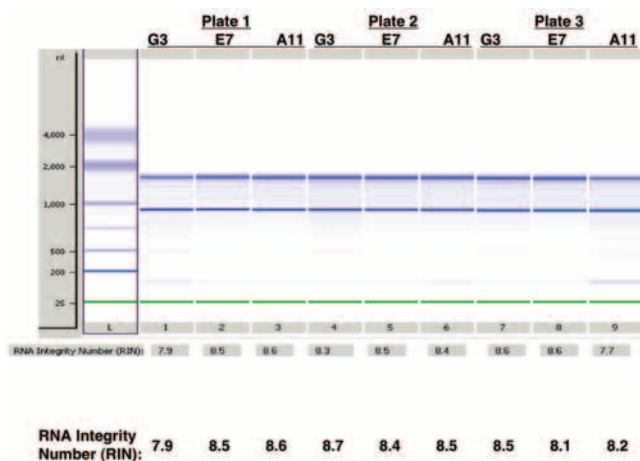
### Comparison of the vacuum and centrifugation protocols

Total RNA was isolated from  $8 \times 10$  mg of mouse liver tissue aliquots as input material for either the RNASpin 96 manual centrifugation or the automated vacuum method. For both methods, the total RNA product was eluted with 100  $\mu$ l of RNase-free water. The eluted volume was 80  $\mu$ l and 100  $\mu$ l for the vacuum and centrifugation protocols, respectively. A sample (20  $\mu$ l) of each eluate was subjected to denaturing gel electrophoresis.

## Results

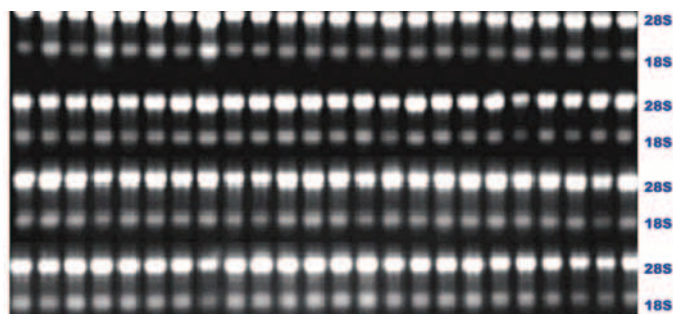
### Consistent isolation of high-quality RNA

Reproducibility is key to the success of any high-throughput process. In this section, we describe several RNA isolation experiments that highlight the consistency of RNA yield and quality well-to-well, plate-to-plate. A common method for measuring total RNA quality and purity is to determine the  $A_{260}/A_{280}$  ratio associated with a specific total RNA sample. The  $A_{260}/A_{280}$  ratio for all the RNA samples described in this section were between 1.9–2.2. 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 96 kit creates an optimal chemical environment for an efficient in-process 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, where contaminating gDNA may interfere and cause spurious product formation and/or low product yield. The efficient in-process DNase I digest of the RNASpin 96 kit effectively eliminated genomic DNA contamination in the preparation of rat liver RNA; hence, the absence of genomic DNA contaminants in the samples shown in Figure 2.



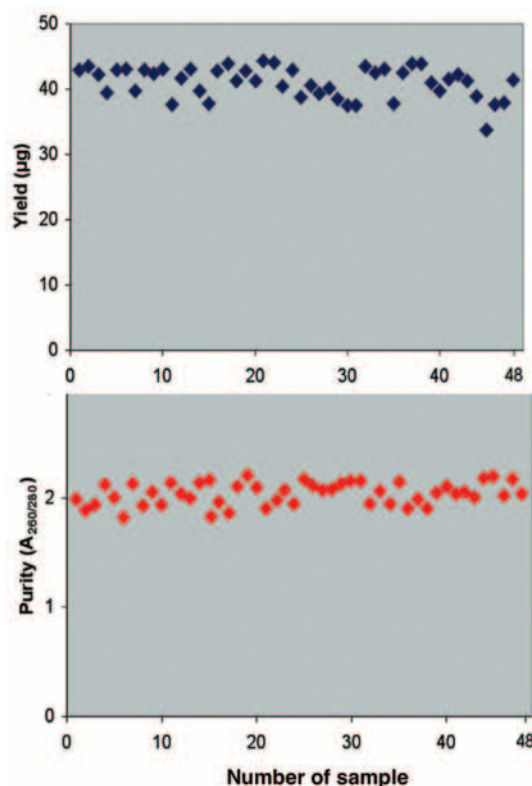
**Fig 2.** The RNeasy 96 kit yields consistent, high-quality total RNA within and across plates. A microliter of RNA sample (out of a total eluate of 100  $\mu$ l) was analyzed on the Agilent 2100 bioanalyzer.

Where the RNA quality was also assessed using the Agilent 2100 bioanalyzer, RNA Integrity Number (RIN) values were on average, greater than 8.0 thereby indicating the presence of high RNA integrity and purity (Fig 2). The rRNA bands were sharp with no visible signs of degradation and the 28S band was about twice as intense as the 18S band (Figs 2 and 3). Vital parameters such as RNA yield, purity, and integrity were all highly reproducible as shown in Figure 4 in which 48 mouse liver samples were prepared in parallel and the yields were  $41.0 \pm 2.4 \mu\text{g}$  (%CV of 5.7), and the  $A_{260}/A_{280}$  ratios were  $2.0 \pm 0.1$ .

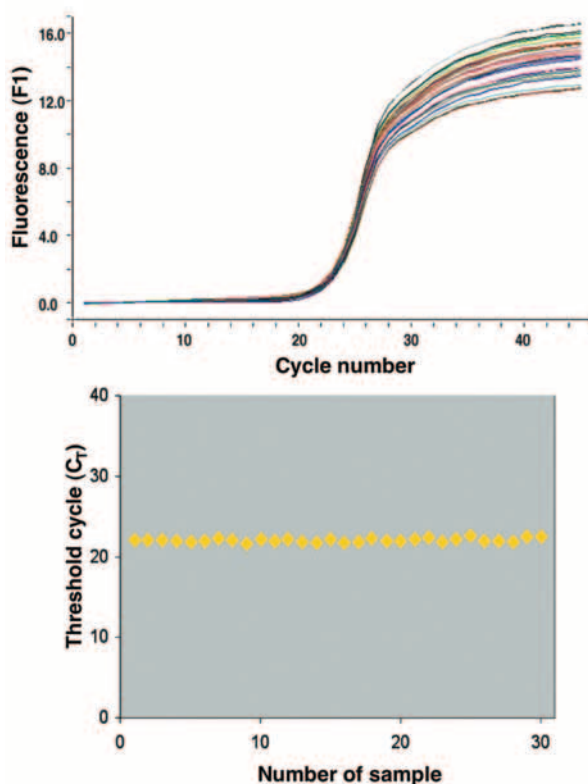


**Fig 3.** Well-to-well reproducibility is maintained using the RNeasy 96 kit for RNA isolation. Twenty microliters of each HeLa cell culture RNA eluate (100  $\mu$ l elution volume) of a 96-well plate was ran in each lane on a denaturing gel.

For qualitative analysis, the isolated RNA was used in a number of rigorous downstream applications: 30 separate, 10-mg mouse liver samples (individual wells) were prepared using the RNeasy 96 Kit. Each sample was then used to interrogate the GAPDH transcript. The LightCycler results shown in Figure 5 indicate that the Cycle Thresholds (CT) were also highly reproducible, with a mean CT of  $22.0 \pm 0.2$  with a %CV of 0.95 (Fig 5).



**Fig 4.** Highly reproducible total RNA yield and purity as determined from  $48 \times 10$ -mg mouse liver tissue samples.



**Fig 5.** The total RNA produced from the RNeasy 96 kit yields consistent performance in sensitive downstream applications. The LightCycler  $C_T$  values from 30 separate total RNA samples are shown.

## Purification of total RNA from large amounts of cells ( $1 \times 10^7$ of input cells)

For many RNA isolation protocols, the processing of large amounts of input cells compromises yield and purity. However, this limitation does not apply to the RNeasy 96 kit because of effective homogenization and the removal of cellular debris with the RNeasy 96 Filter Plate. Thus, the RNeasy 96 kit can be used to process as much as  $1 \times 10^7$  cells with high yield and purity: the  $A_{260}/A_{280}$  ratio for all the RNeasy samples was between 1.94 and 1.96 (Fig 6).

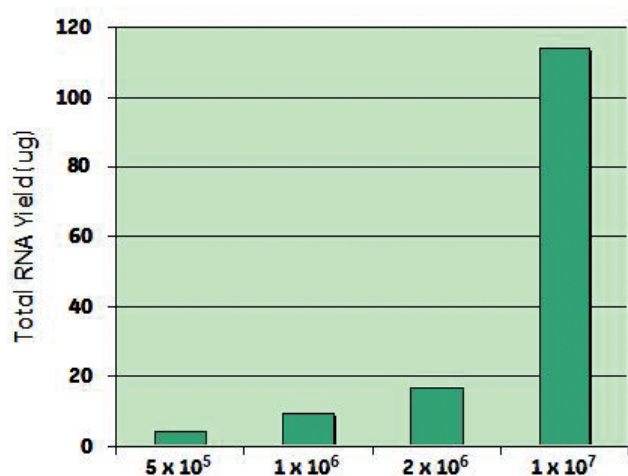


Fig 6. Successful isolation of total RNA with the RNeasy 96 kit from up to  $1 \times 10^7$  for HeLa cells.

## Purification of total RNA from a relatively small amount of cells ( $\leq 10$ cells)

RT-PCR results using total RNA isolated from a range of HeLa cell amounts are shown in Figure 7. The GAPDH RT-PCR product was easily detected from as a low a sample size as 10 HeLa cells. This makes the RNeasy 96 kit particularly suitable for the isolation of high-quality RNA from very small amounts of input cells.

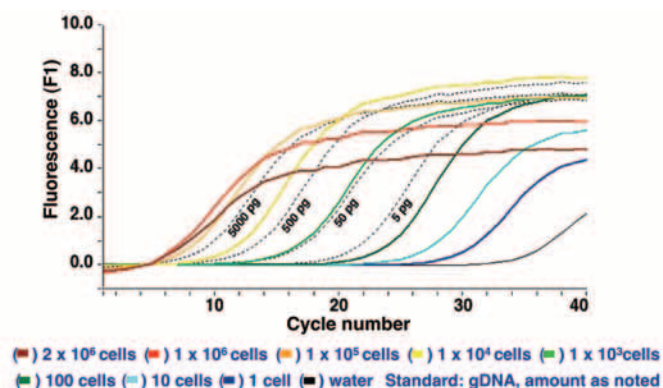


Fig 7. The efficient isolation of RNA with the RNeasy 96 kit allows for the detection of amplifiable RNA from very small amounts of cells.

## Detection of gene expression by Northern analysis

GAPDH gene expression is often used as a positive expression control in gene expression profiling. Therefore, GAPDH is expected to be expressed at a detectable level in many cell types, including cultured HeLa cells. The known pattern of GAPDH gene expression in cultured HeLa cells is shown for 48 separate, 96-well culture samples (Fig 8). This data highlights the reproducibility aspect of the RNeasy 96 kit and also shows that it can be used to isolate sufficient, high-quality total RNA for Northern blot analysis.

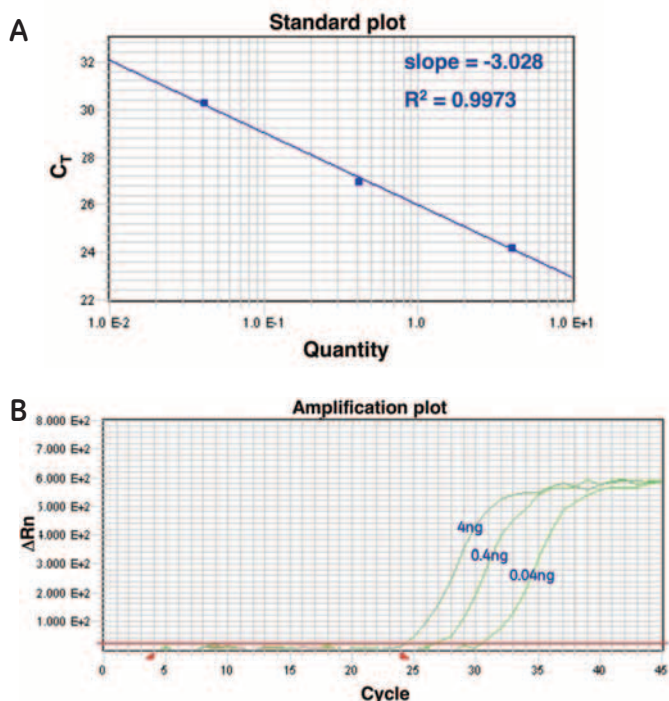


Fig 8. Northern blot analysis of the GAPDH transcript in 48 separate HeLa cell cultures. Fifteen microliters of each HeLa cell culture RNA eluate from a 96-well plate was run per lane (see Fig 3 legend for additional details).



## Detection of RNA transcripts by QRT-PCR

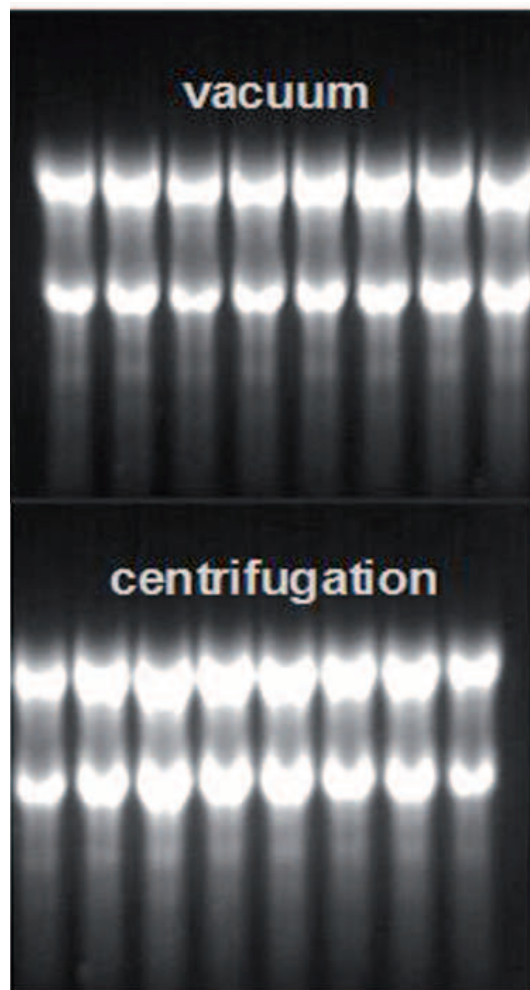
The rat thyroid hormone sulfotransferase, encoded by the *ST1B1* gene, is an important regulator of thyroid hormone metabolism. As with other important transcripts, measurement of the relative expression level in a given tissue (liver in this case) is important for understanding its biological function. The data presented here shows that the quality of the RNA generated from the RNAspin 96 kit is high enough for sensitive downstream applications like QRT-PCR (Fig 9).



**Fig 9.** Total RNA from the RNAspin 96 kit performed well in sensitive downstream applications like QRT-PCR. Titration of an RNAspin 96 kit rat liver total RNA sample (NAP303) 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-PCR CT curves showing typical response curves for clean RNA template.

## A comparative analysis of centrifugation and vacuum methods

The ability to use the RNAspin 96 kit with either a benchtop centrifuge or on an automated platform enhances the flexibility of the kit. A comparison of the RNA output (Fig 10) from both the centrifugation and vacuum methods indicates that although the RNA yields were similar, the centrifugation method produced slightly higher yields (about an 8% increase, on average).



**Fig 10.** Comparative analysis of isolated total RNA via centrifugation and vacuum protocols on a denaturing gel with 20 µl of each eluate per lane.

## Conclusions

The RNAspin 96 RNA Isolation kit reproducibly (well-to-well, plate-to-plate) yields total RNA of high quality and integrity with little or no contaminating genomic DNA. The RNA product can be purified in varying amounts from a diverse range of sample types including samples that are difficult to process. Typical input tissues include rat and mouse tissue (liver, kidney, brain), different human cell lines, and *Streptococci*, *Actinobacillus pleuropneumoniae* and *Pseudomonad species*. The quality, integrity, and yield of total RNA is high enough for the most sensitive of downstream applications such as QRT-PCR and Northern Blot.

## 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).

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