Purification of Concentrated, High-Quality RNA from Cells and Tissues

ReliaPrep™ RNA Cell and Tissue Miniprep Systems

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Products Featured:

- ReliaPrep™ RNA Cell Miniprep System
- ReliaPrep™ RNA Tissue Miniprep System

Using the ReliaPrep™ membrane-based systems, 1×10^2–5×10^6 cultured cells or 0.25–20mg tissue can be processed per purification

The ReliaPrep™ RNA Miniprep Systems offer a complete solution for the isolation of quality RNA from very small amounts of cultured cells or tissues. They provide a rapid purification method with the option of elution in a small volume (15µl or less) to generate highly concentrated RNA. Purification is achieved without using phenol/chloroform extraction or beta-mercaptoethanol, and a simple DNase step is included to eliminate genomic DNA efficiently. In this article, we demonstrate that RNA isolated from cells and tissues using the ReliaPrep™ Systems is intact, highly pure and ideally suited for applications such as RT-qPCR.

Introduction

Regulation of gene expression continues to be an area of high interest for both academic researchers and biopharmaceutical companies. While there are numerous methods for determination of the upregulation or suppression of gene expression, most begin with isolation and purification of RNA.

The ReliaPrep™ RNA Cell Miniprep System provides a fast and simple technique for purifying intact total RNA from cultured cells in as little as 30 minutes, depending on the number of samples processed. The manual, column-based method has several advantages over most commercially available kits. The novel column matrix allows for high capacity on a compact resin bed situated in the narrow throat region. Because all the available RNA passes through this small aperture, the column can bind and purify RNA from minimal sample inputs. This design greatly improves wash efficiency for bound RNA, while allowing elution in 15µl or less. The columns have virtually no hold volume so recoveries are outstanding. The ReliaPrep™ System also incorporates an on-column DNase treatment designed to substantially reduce genomic DNA contamination, which can interfere with downstream amplification-based methods. Purification is achieved without the use of beta-mercaptoethanol, phenol:chloroform extraction or ethanol precipitation.

The ReliaPrep™ RNA Tissue Miniprep System is based on the same column technology and is designed to purify RNA from animal tissues. The reagents in the tissue system are altered to address the more complex composition of tissue sample types but the advantages remain the same, and as little as 0.25mg of tissue can be used for successful isolation of highly pure RNA. The tissue system allows use of a variety of tissue types without significant protocol changes or

lengthy enzymatic treatments to remove proteins from fibrous tissues or lipids from adipose tissues. It may no longer be necessary to order separate kits for these more difficult tissue types. Elution is still accomplished with low volumes of eluate, allowing purification of highly concentrated RNA from minimal sample inputs. This has particular advantages in RT-qPCR where template volumes often must be limited to allow for addition of other reagents.

Direct Purification of RNA

Successful isolation of intact RNA requires four steps:

1. Effective disruption of cells or tissue.
2. Denaturation of nucleoprotein complexes.
3. Inactivation of endogenous ribonuclease (RNase) activity.
4. Removal of contaminating DNA and proteins.

The most important step is the inactivation of endogenous RNases, which are released from membrane-bound organelles upon cell disruption. The ReliaPrep™ RNA Cell Miniprep System combines the disruptive and protective properties of guanidine thiocyanate (GTC) and 1-Thioglycerol to inactivate the ribonucleases present in cell extracts. GTC disrupts nucleoprotein complexes, allowing the RNA to be released into solution and isolated free of protein. Nucleic acids in lysates are bound to the ReliaPrep™ Minicolumns by centrifugation. The binding reaction occurs rapidly due to disruption of water molecules by chaotropic salts, favoring adsorption of nucleic acids to the column. RNase-free DNase I is applied directly to the membrane to digest contaminating genomic DNA. The bound total RNA is further purified from contaminating salts, proteins and cellular components by simple washing steps. Finally, total RNA is eluted from the membrane using Nuclease-Free Water. This procedure yields an essentially pure fraction of RNA after a single round of purification without organic extractions or precipitations. RNAs smaller than 200 nucleotides are excluded. The procedure is easy to perform with small quantities of cultured cells, and it can be used to process multiple samples.

ReliaPrep™ RNA Cell Miniprep System

Processing Capacity

The ReliaPrep™ RNA Cell Miniprep System is optimized for total RNA isolation from a range of input cell numbers, from 1×10^2 to 5×10^6 cultured mammalian cells with a broad spectrum of RNA expression levels.

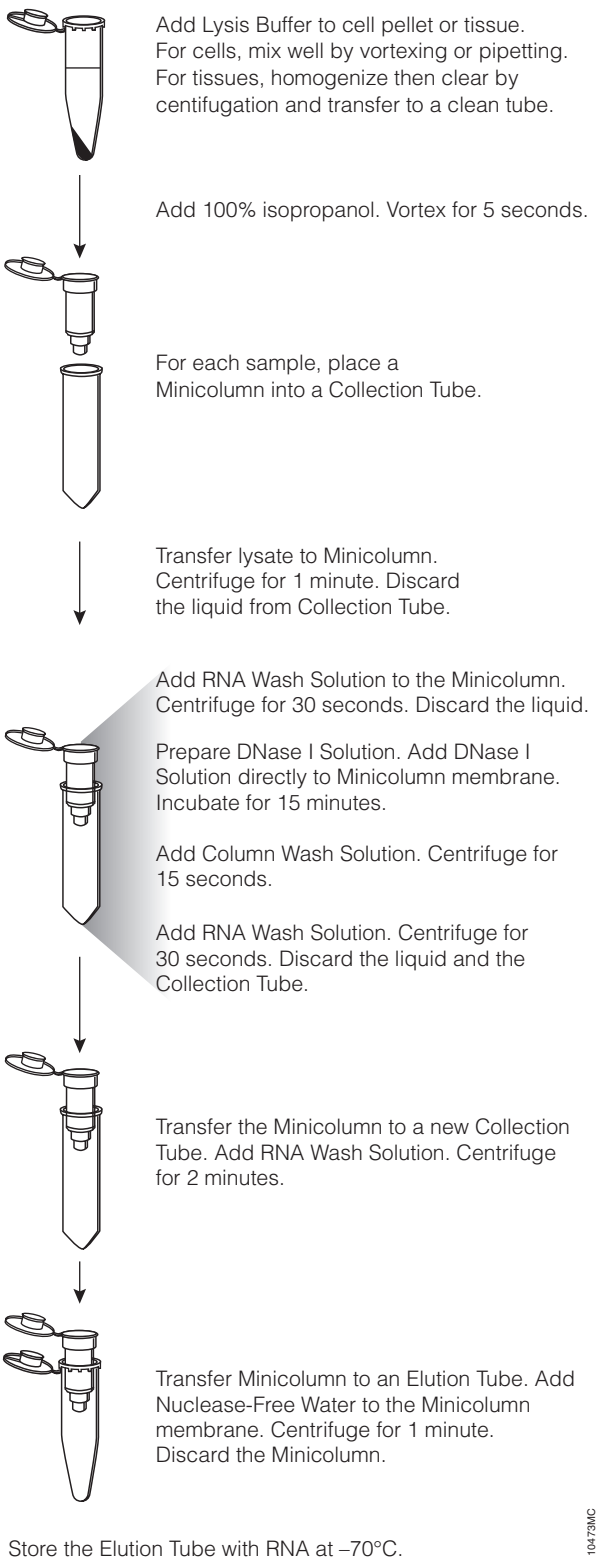


Figure 1. ReliaPrep™ RNA Miniprep Systems Protocol Overview.

Downstream Applications

RNA purified with the ReliaPrep™ RNA Cell Miniprep System is suitable for many molecular biology applications, including RT-PCR, microarrays and Northern blot hybridizations. For all downstream applications, you should continue to protect your samples from RNases by wearing gloves and using solutions and centrifuge tubes that are RNase-free. Additionally, the use of RNasin® Ribonuclease Inhibitor (Cat.# N2511) is recommended to protect the purified RNA samples from degradation by RNases introduced from the environment.

RNA Integrity

RNA purified using the ReliaPrep™ RNA Cell Miniprep System is of high integrity, as illustrated in Figure 2. Twelve RNA preparations purified from 10^5 HeLa cells were tested on the Agilent Bioanalyzer 2100 instrument using an RNA Nano 6000 chip and protocol. The integrity of the purified RNA is indicated by a 10-point RIN scale, typically RIN values above 8.0 are considered suitable for a wide range of applications.

Reproducibility of Yield and Purity

The success of any RNA purification system begins with reproducibility. Figure 3 shows the yields obtained from a set of 18 HeLa cell RNA preps performed simultaneously.

While particular care should be taken to rapidly lyse and load samples onto the columns, the data in Figures 2 and 3 show that timing is not a critical factor, even when processing a large sample set. Purity and yield are unaffected.

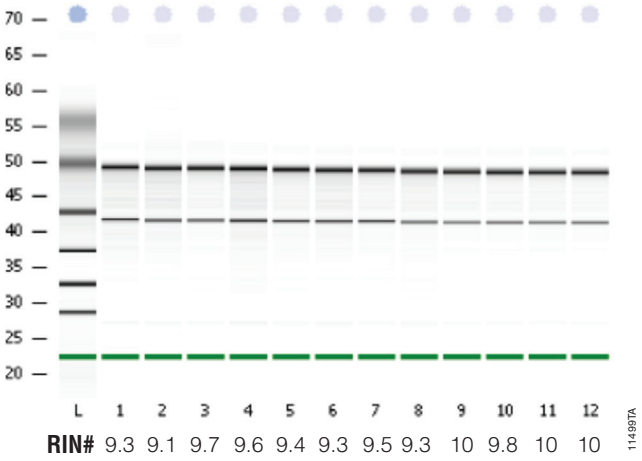


Figure 2. Quality of RNA purified using ReliaPrep™ RNA Cell Minipreps. RNA isolated from 1 x 10^5 HeLa cells was analyzed using a Agilent Bioanalyzer 2100 instrument with a RNA nano 6000 chip. RNA was eluted in a volume of 15µl.

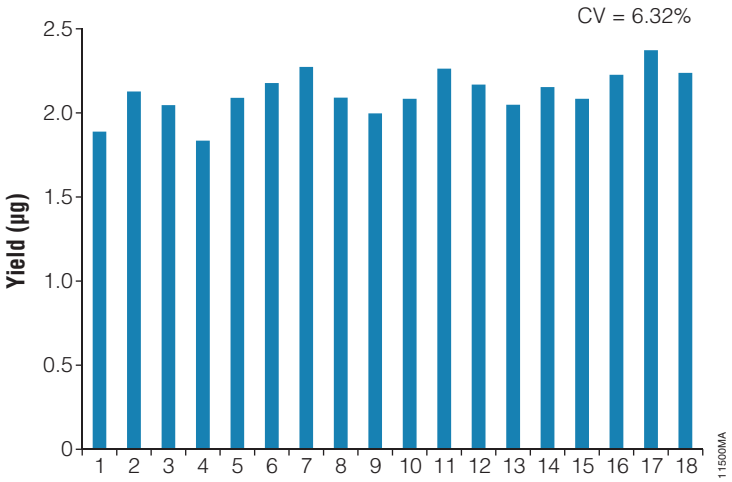


Figure 3. RNA yield from 18 simultaneous purifications from HeLa cells. HeLa Cell RNA was isolated from 18 samples in parallel using the ReliaPrep™ RNA Cell Miniprep System. RNA was eluted in 15µl and yield was determined by absorbance spectroscopy. Average yield was 2.12µg and the CV was 6.32%.

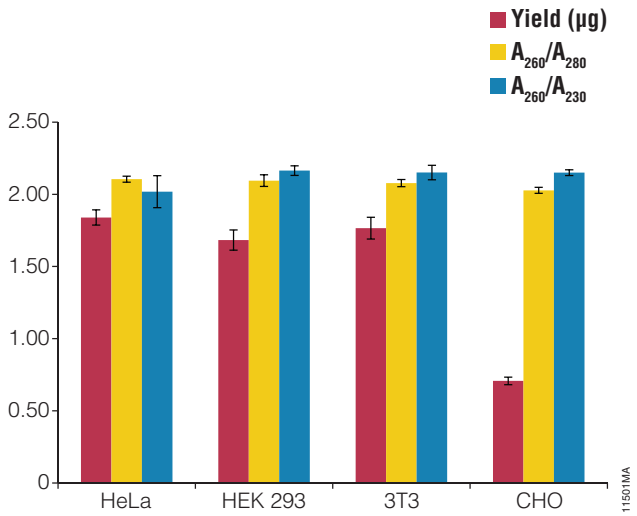


Figure 4. RNA yield and purity from various cell types using the ReliaPrep™ RNA Cell Miniprep System. Total RNA was isolated in triplicate from 100,000 HeLa, HEK293, 3T3 or CHO cells. One microliter of purified RNA was analyzed by absorbance spectroscopy using a NanoDrop® 1000 instrument.

This consistency is not unique to HeLa cell RNA preparations. Figure 4 shows the results of 12 individual purifications from several commonly used cell culture lines, demonstrating both the consistency and purity commonly obtained using the ReliaPrep™ Cell Miniprep System. It is important to note that many factors affect RNA yield and integrity, including cell number, cell type, growth conditions and others.

Elution Volume

While the standard ReliaPrep™ Cell Miniprep System protocol calls for elution in 15µl for purifications from as much as 5×10^5 cultured cells, it is possible to use considerably less if a higher concentration of RNA is desired. Figure 5 shows that extremely concentrated RNA can be obtained when eluting in volumes as small as 7µl, however yield does decrease with decreasing elution volume.

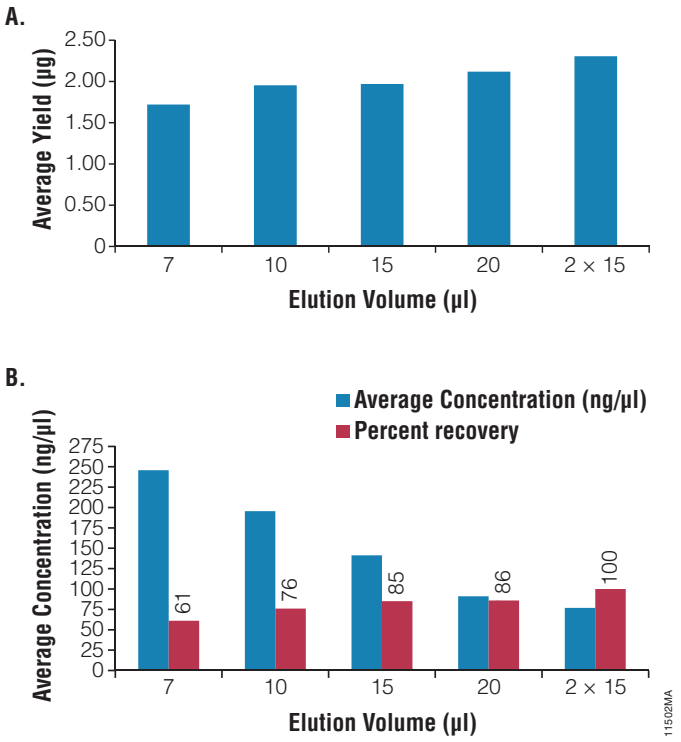


Figure 5. Yield and concentration of RNA with varying elution volume. RNA was isolated from 100,000 HeLa Cells and eluted with various volumes from 7–30 (2 × 15) µl. **Panel A**. Average yield. **Panel B**. Average concentration. Recovery was measured by absorbance spectroscopy.

Effect of Cell Harvesting Method

Typically, mammalian cell culture uses a variety of dish and flask formats, and adherent cells are often harvested by trypsinization prior to processing. Figure 6 shows the RNA yields obtained after trypsin harvesting or direct lysis in various dishes or flasks. For small cell numbers, both methods resulted in similar recoveries. For larger plates and flasks, trypsin treatment resulted in greater recovery of RNA from the samples. Purity of recovered RNA was unaffected (data not shown).

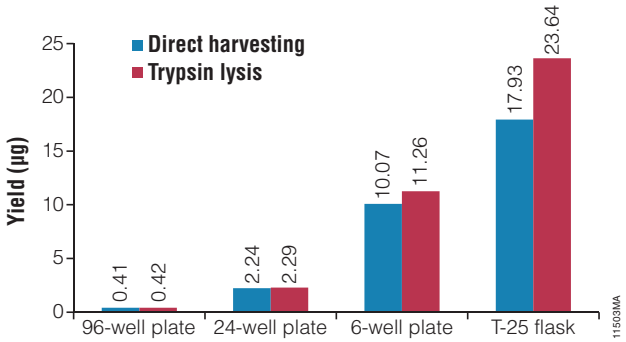


Figure 6. Effect of cell harvesting methods on RNA yield. Tissue culture cells grown in various dishes were washed with PBS and then either lysed directly or trypsinized and then lysed. RNA was quantitated by absorbance spectroscopy using a Nanodrop® 1000 instrument. 96-well and 24-well plates were lysed using 100µl/well, 6-well plates using 250µl, and T-25 flasks using 500µl.

Elution in low volumes, allows purification of highly concentrated RNA from minimal sample inputs

ReliaPrep™ RNA Tissue Miniprep System

Processing Capacity and RNA Integrity

The ReliaPrep™ RNA Tissue Miniprep System is optimized for total RNA isolation from as little as 0.25mg and up to 20mg of animal tissue. Unlike many commercially available kits, the ReliaPrep™ RNA Cell Miniprep System provides reagents suitable for processing non-fibrous or fibrous tissues. Processing is accomplished without the need for lengthy protease treatments or elevated incubation temperatures, which can adversely affect RNA integrity. The simple protocol requires the user to estimate sample size as either less than 5mg or greater than 5mg for reagent volume use. Lysis using a tissue homogenizer and a short centrifugation to remove debris is all that is required before beginning purification. Figure 7 shows Agilent Bioanalyzer data from samples purified from various tissue types using the ReliaPrep™ RNA Tissue Miniprep System. Intact RNA was isolated from input tissue amounts from 5 to 20 mg for all sample types, as evidenced by RIN values ranging from 8.4 to 10.

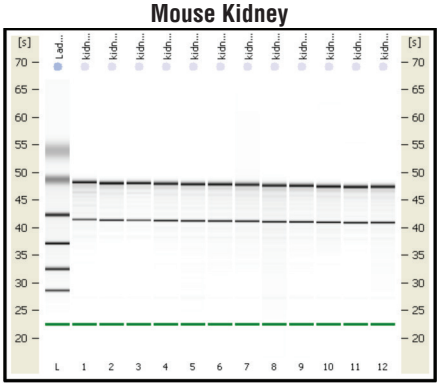
Reproducibility of Yield and Purity

The ReliaPrep™ RNA Tissue Miniprep System shows excellent reproducibility of results. Table 1 shows the data from three experimental runs using 18 samples each from three different tissue types. Yield and purity were determined by absorbance spectroscopy using a Nanodrop® 1000 instrument. The average yield and purity and standard deviation were determined to assess the reproducibility of the method.

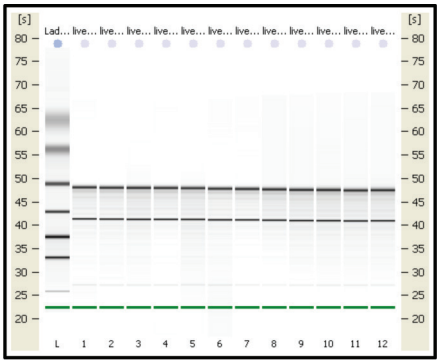
Table 1. Yield and Purity of RNA From 18 Simultaneously Processed Samples.

Tissue	Yield			A_{260}/A_{230}		
	Value	Std. Dev.	%CV	Value	Std. Dev.	%CV
Mouse Kidney	18.55	0.83	4.50	2.13	0.15	7.10
Mouse Liver	30.63	2.336	7.60	2.16	0.078	3.60
Mouse Spleen	10.75	1.3	12.10	1.99	0.14	7.20

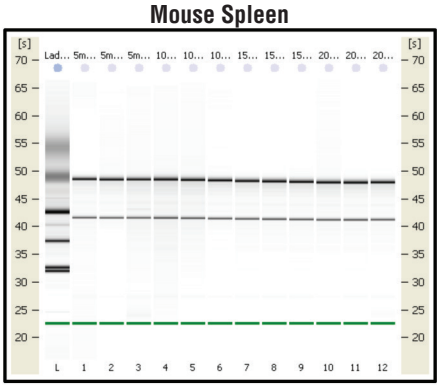
A. Mouse Kidney



B. Mouse Liver



C. Mouse Spleen



D. RIN Values

Tissue	5mg			10mg			15mg			20mg		
Mouse kidney	9.1	9.5	9.9	9.2	9.0	8.9	8.8	8.3	9.3	9.0	9.5	8.8
Mouse liver	8.4	8.0	8.6	8.0	8.5	8.6	8.7	8.5	8.5	8.7	8.5	8.7
Mouse spleen	9.6	10	8.4	8.1	8.6	10	9.1	9.5	9.3	9.7	9.5	9.5

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Figure 7. Quality of RNA isolated from various mouse tissues. RNA was isolated from 5–20mg of mouse kidney (A), liver (B) or spleen (C). RNA was analyzed using a Agilent Bioanalyzer 2100 instrument with a RNA nano 6000 chip. Samples were processed in triplicate. RIN values obtained for all samples were above 8.0 (D).

Figure 8 shows the results of RNA purification from a variety of tissue types. Soft and non-fibrous tissues like brain, spleen, kidney, liver or pancreas are processed using the non-fibrous protocol. Fibrous tissues like heart and lung use an alternate protocol requiring just one additional reagent (included with the kit) and brief centrifugation to remove interfering proteins and collagen. Both protocols are provided in the ReliaPrep™ RNA Tissue Miniprep System Technical Manual #TM365. Regardless of the tissue type, these two protocols provide consistent purification of high-quality RNA.

As described for the ReliaPrep™ RNA Cell Miniprep System, users of the ReliaPrep™ RNA Tissue Miniprep System can also choose standard elution volumes (15µl for less than 5mg sample input and 30µl for greater than 5mg sample input) or can vary the elution scheme to obtain more concentrated RNA or to maximize yield. If the expected yield is greater than 30µg, best results are obtained using a two-step elution as shown in Figure 9.

Downstream Application: RT-qPCR

The most common downstream application for purified RNA is RT-qPCR. Therefore, it is important to know whether the RNA preparation has significant carryover of inhibitory contaminants. In the experiment shown in Figure 10, HeLa RNA was used as template for RT-qPCR using the Promega GoTaq® 1-Step RT-qPCR System with 25ng of template per reaction. Triplicate test reactions were then spiked with 1, 5, or 9 µl of mouse lung RNA (0.31µg/µl). Human GAPDH primers at 0.2µM were used for amplification and the total reaction volume was 25µl. The results show that even when almost 2.8µg of contaminating RNA was added (9µl mouse lung), only marginal inhibition was observed.

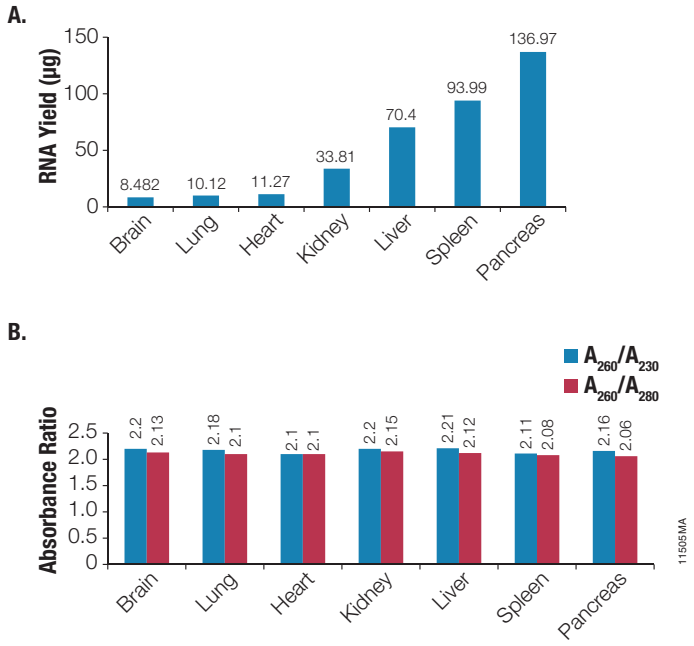
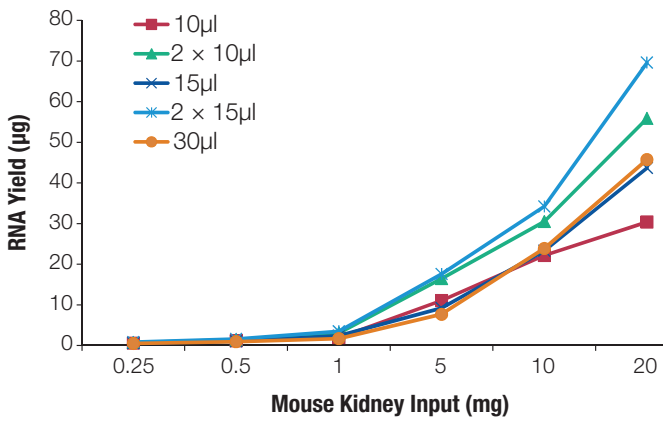


Figure 8. Typical RNA yields from various mouse tissues. RNA was isolated from 10mg samples of various mouse tissues using the ReliaPrep™ RNA Tissue Miniprep System. Yield and purity were determined by absorbance spectroscopy using a Nanodrop® 1000 instrument.



Input (mg)	Yield					
	10µl	2 x 10µl	15µl	2 x 15µl	30µl	2 x 30µl
0.25	0.61	0.72	0.58	0.86	0.51	0.51
0.5	1.14	1.39	1.05	1.61	0.93	0.93
1.0	1.94	3.06	2.41	3.50	1.70	1.88

Figure 9. Effect of elution on yield of RNA from mouse kidney. RNA was isolated from increasing amounts of kidney tissue using various elution schemes. Yield was determined by absorbance spectroscopy using a Nanodrop® 1000 instrument. The table shows the yield from 0.25, 0.5 and 1mg tissue amounts.

Conclusion

Promega offers two new RNA purification platforms: The ReliaPrep™ RNA Cell Miniprep System purifies RNA from cell culture samples and the ReliaPrep™ RNA Tissue Miniprep System purifies RNA from animal tissues. Both systems use the new ReliaPrep™ column, which has the advantage of reduced elution volume, more efficient removal of contaminants, and higher purity of eluted RNAs. Both systems also require lower sample inputs while providing simplified protocols with no requirement for additional reagents.

Ordering Information

Product	Size	Cat.#
ReliaPrep™ RNA Cell Miniprep System	10 preps	Z6010
	50 preps	Z6011
	250 preps	Z6012
ReliaPrep™ RNA Tissue Miniprep System	10 preps	Z6110
	50 preps	Z6111
	250 preps	Z6112

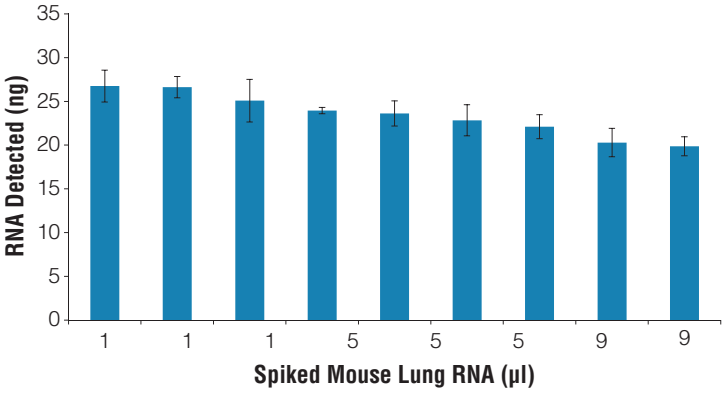


Figure 10. Inhibition of RT-qPCR by ReliaPrep™ RNA Tissue miniprep eluates. Human-specific primers were used to amplify GAPDH from a fixed amount of human RNA. Mouse RNA (1, 5 or 9 µl) isolated with the ReliaPrep™ RNA Tissue Miniprep System was spiked into each reaction. The GAPDH concentration was determined using a purified HeLa RNA standard.

ReliaPrep™ columns have the advantage of reduced elution volume, more efficient removal of contaminants, and higher purity of eluted RNA

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