

# RNA Purification

## Spectrum™ Plant Total RNA Kit

The Spectrum Plant Total RNA Kit employs a new lysis and binding chemistry and a convenient column-based 'bind-wash-and-elute' format to purify up to 100 µg of total RNA from 100 mg of tissue in about 30 minutes. Typical yields range from 20-60 µg.

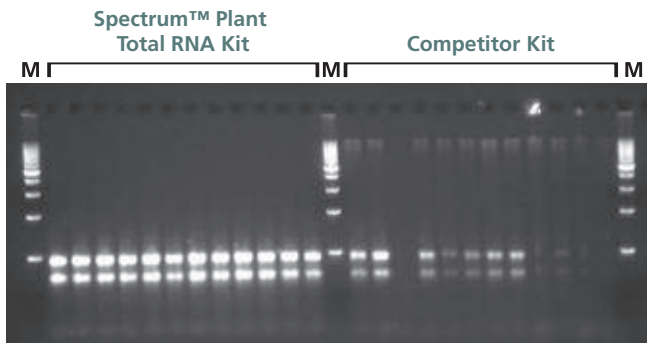
After grinding tissue to a fine powder in liquid nitrogen, cells are lysed and cellular debris is physically and chemically separated from endogenous RNA. RNA is then bound to a column supported silica substrate and several wash steps remove remaining contaminants. Lastly, total RNA is eluted from the column and used in typical applications, such as Northern blots, and RT- and qRT-PCR.

### Features and Benefits

- Specially designed for research with difficult plant tissues
- Yields up to 60 µg of pure concentrated RNA per prep
- Efficient protocol allows for RNA purification in 30 minutes or less

**Storage:** Room Temperature

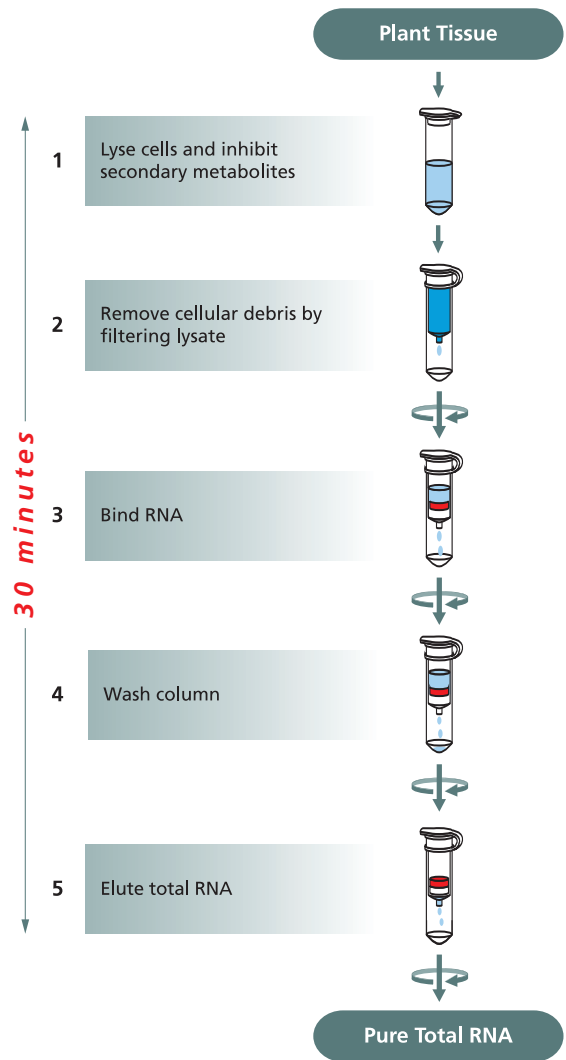
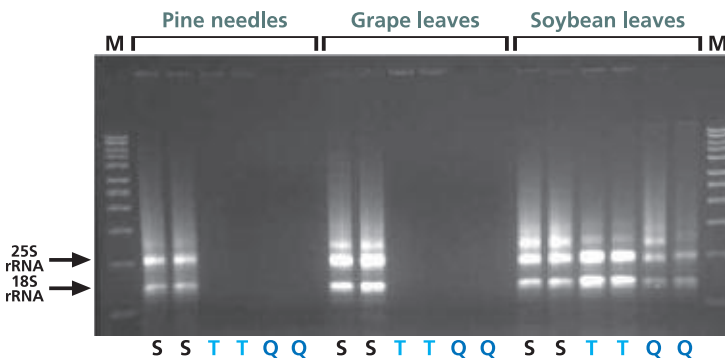
R: 22-24-34-51/53 S: 26-36/37/39-45-60



### Total RNA purification from maize seed endosperm

**Figure 1.** Total RNA was purified from maize seed endosperm (14 days after pollination). The samples loaded in each lane were adjusted to 0.2 µg/µl, based on spectrophotometric absorbance data.

Data kindly provided by Moises Cortes-Cruz, Ph.D. while in the Messing lab at The Waksman Institute (Rutgers University).



### Agarose gel analysis of total RNA purifications

**Figure 2.** Each sample type was processed in duplicate using the Spectrum Plant Total RNA Kit (black "S" lanes), a phenol/guanidine isothiocyanate solution and chloroform extraction (light blue "T" lanes), or a commonly available plant total RNA purification kit (dark blue "Q" lanes). Molecular marker (black "M" lanes) is a 1 kb DNA ladder.

Pine samples: Each lane was loaded with 2% of total recovery (S samples) or 10% of total recovery (T and Q samples).  
Grape samples: Each lane was loaded with 1.5% of total recovery (S samples) or 10% of total recovery (T and Q samples).  
Soybean samples: Each lane was loaded with 1.5% of total recovery. (Recovery determination for all samples was calculated using spectrophotometric absorption data)

### Ordering Information

Cat. No.	Product Description	Preps	Quantity
<b>STRN10</b>	Spectrum™ Plant Total RNA Kit	10	1 kit
<b>STRN50</b>	Spectrum™ Plant Total RNA Kit	50	1 kit
<b>STRN250</b>	Spectrum™ Plant Total RNA Kit	250	1 kit

# RNA Purification

## GenElute™ Mammalian Total RNA Miniprep Kits

### For isolation of total RNA from mammalian cells and tissues

The GenElute Mammalian Total RNA Miniprep Kit combines silica-membrane technology with a convenient spin column format for a rapid bind, wash, and elute method to prepare high quality total RNA.

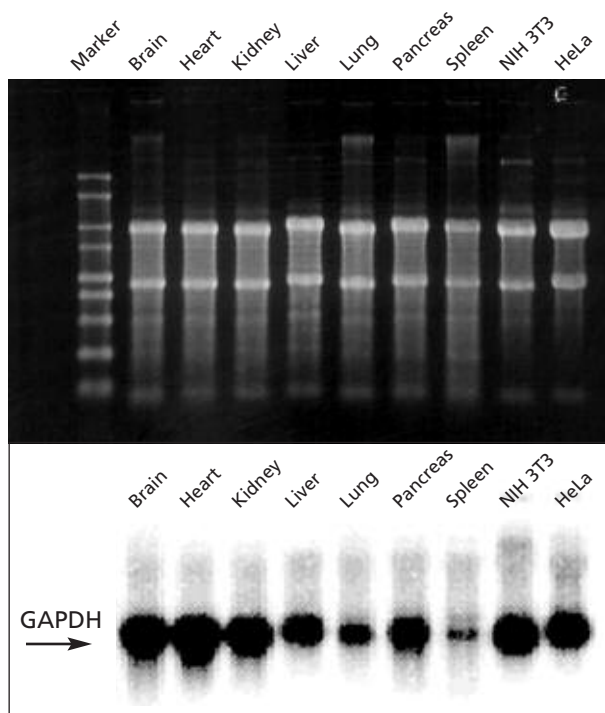
Samples are lysed and homogenized in guanidine thiocyanate and 2-mercaptoethanol to release RNA and inactivate RNases. Lysates are spun through a filtration column to remove cellular debris and shear DNA. The filtrate is then applied to a high capacity silica column to bind total RNA, followed by washing and elution. Up to 150 µg of total RNA can be recovered per prep in 100 µl of water. The purified RNA is ready for Northern blots (Fig. 1), RT-PCR (Fig. 2) and other common applications.

### Features and Benefits

- Purifies total RNA from up to 10<sup>7</sup> cells or 40 mg of tissue per prep
- Yields up to 150 µg of pure, concentrated total RNA per prep
- Recover RNA from as few as 100 cells
- Simple and efficient – 12 to 18 preps in 30 minutes
- Faster than gravity flow anion exchange methods
- No cumbersome steps associated with resins and magnetic slurries
- 40% more purifications than the leading supplier

**Storage:** Room Temperature

R: 24-20/22-41-37/38 S: 53-45-26-36/37/39



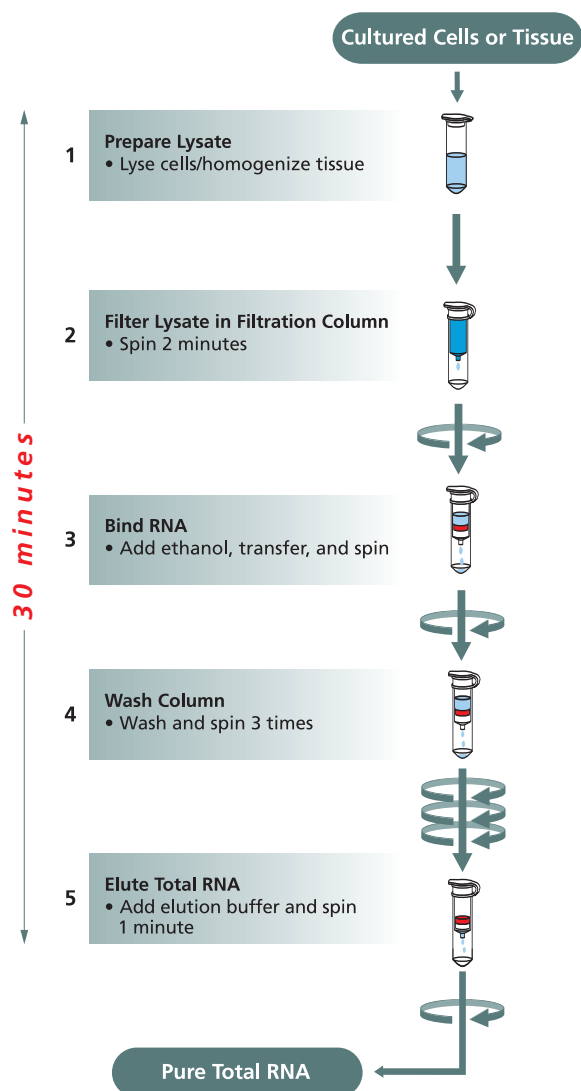
### High Quality RNA from various tissues and cells

Figure 1A. Formaldehyde-agarose gel and Northern blot of total RNA purified with GenElute™ Mammalian Total RNA Miniprep Kit.

**Upper panel:** 2 µg of each RNA analyzed on a 1.2% agarose gel containing 0.6 M formaldehyde.

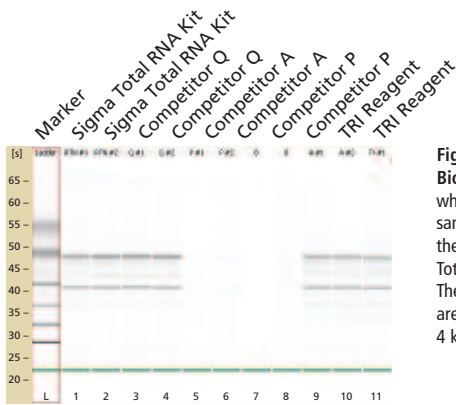
**Lower panel:** Corresponding Northern blot hybridized with a <sup>32</sup>P-labeled RNA probe for GAPDH in PerfectHyb™ Plus hybridization buffer (Cat. No. H7033).

**Note:** The GAPDH probe detected a single mRNA band in every lane with little or no smearing. Even RNA from pancreas, which is known to have high RNase levels, is not degraded.

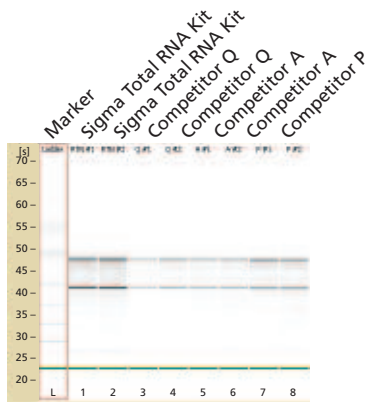


# RNA Purification

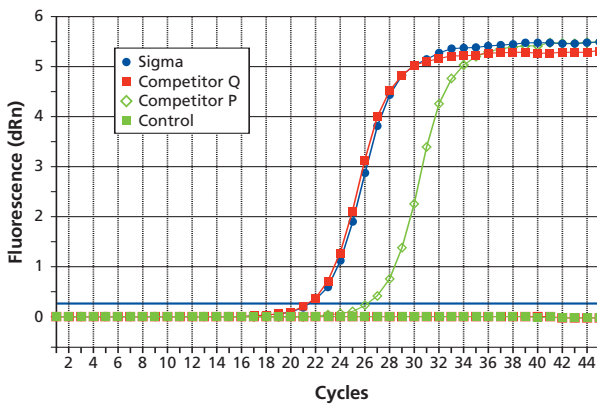
## High Quality RNA from various tissues and cells



**Figure 1B. Agilent 2100 Bioanalyzer data.** Human whole blood and total RNA samples were purified using the GenElute Mammalian Total RNA Miniprep Kit. The 18S and 28S bands are clearly visible at 2 and 4 kb, respectively.



**Figure 1C. Agilent 2100 Bioanalyzer data.** Mouse liver tissue and total RNA samples were purified using the GenElute Mammalian Total RNA Miniprep Kit. The 18S and 28S bands are clearly visible at 2 and 4 kb, respectively.



**Figure 2A. Quantitative RT-PCR of total RNA isolated from human whole blood.** Total RNA was purified with the GenElute Mammalian Total RNA Miniprep Kit. Two-step qRT-PCR was performed using an MMLV reverse transcriptase and a specially formulated Hot Start SYBR Green PCR ReadyMix. The quantitative PCR step was run in the Stratagene Mx3000P using 169 base pair General transcription factor IIIA (GTF3A) target. The assay was run at 94 °C for 3 minutes, followed by 2-step cycling conditions for 45 cycles at 94 °C for 15 seconds, and 60 °C for 1 minute.

**Table 1. Yield from HeLa Cells**

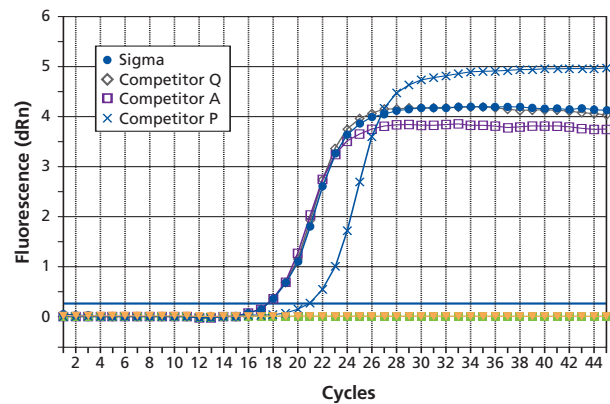
HeLa cells 7,000,000	Average ng/ml yield from Bioanalyzer	Average RIN
Sigma	904.5	9.9
Supplier Q	904.5	9.9
Supplier A	888.0	9.9
Supplier P	178.5	10.0

**Table 2. Yield from Mouse Liver**

Mouse Liver tissue ~30 mg	Average ng/ml yield from Bioanalyzer	Average RIN
Sigma	1200.0	8.3
Supplier Q	427.5	8.8
Supplier A	537.0	7.9
Supplier P	810.0	8.0

**Table 3. Yield from Whole Blood**

Whole blood 1.5 ml	Average ng/ml yield from Bioanalyzer	Average RIN
Sigma	31.5	9.7
Supplier Q	33.0	9.7
Supplier P	4.0	8.1



**Figure 2B. Quantitative RT-PCR of total RNA isolated from human HeLa cells.** Total RNA was purified with the GenElute Mammalian Total RNA Miniprep Kit. Two-step qRT-PCR was performed using an MMLV reverse transcriptase and a specially formulated Hot Start SYBR Green PCR ReadyMix. The quantitative PCR step was run in the Stratagene Mx3000P using 169 base pair General transcription factor IIIA (GTF3A) target. The assay was run at 94 °C for 3 minutes, followed by 2-step cycling conditions for 45 cycles at 94 °C for 15 seconds, and 60 °C for 1 minute.

## Ordering Information

Cat. No.	Product Description	Preps	Quantity
<b>RTN10</b>	GenElute™ Mammalian Total RNA Miniprep Kit	10	1 kit
<b>RTN70</b>	GenElute™ Mammalian Total RNA Miniprep Kit	70	1 kit
<b>RTN350</b>	GenElute™ Mammalian Total RNA Miniprep Kit	350	1 kit

# RNA Purification

## GenElute™ Bacterial Total RNA Miniprep Kit

Sigma's GenElute Bacterial Total RNA Purification Kit combines silica-membrane technology with a convenient spin column format for a rapid bind, wash, and elute method to prepare high quality total RNA from both Gram-negative and Gram-positive bacteria.

Cells are lysed and homogenized in a guanidine thiocyanate-containing buffer to ensure thorough denaturing of macromolecules and inactivation of RNases. Addition of ethanol causes RNA to bind when the lysate is spun through a silica membrane in a microcentrifuge tube. After washing to remove contaminants, total RNA is then eluted. The purified RNA is ready for reverse transcription, PCR, labeling, microarray analysis, and other common applications.

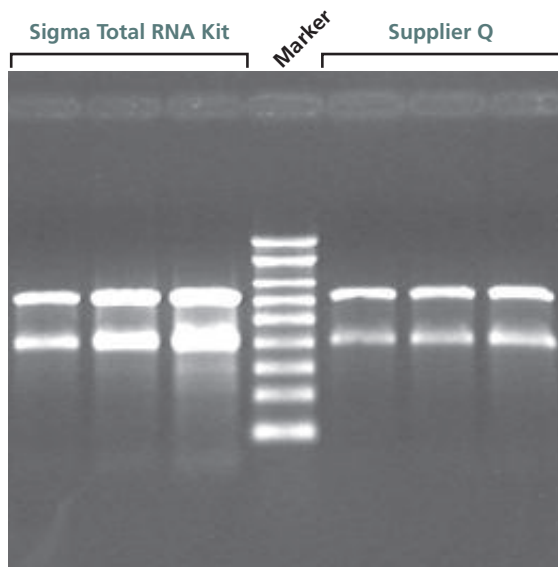
### Features and Benefits

- Purity – high quality RNA in less than one hour
- Flexible – suitable for both Gram-positive and Gram-negative bacteria
- Robust – yields of 30-55 µg
- Efficient – purified RNA is ready for RT-PCR, Northern blot, and microarray analysis

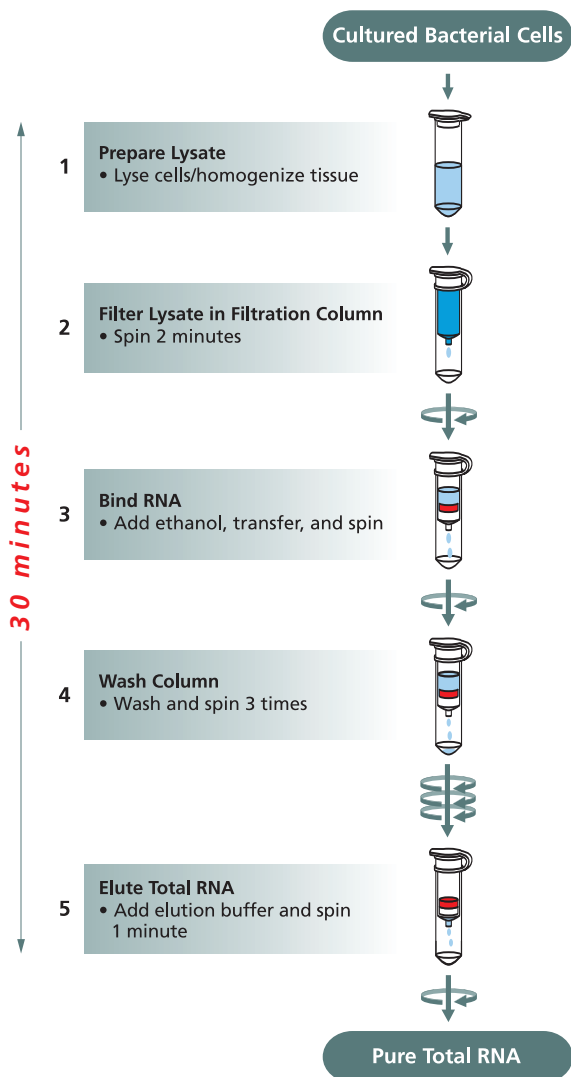
**Storage:** Room Temperature

R: 20/22-24-34-51/53 S: 26-36/37/39-45-60

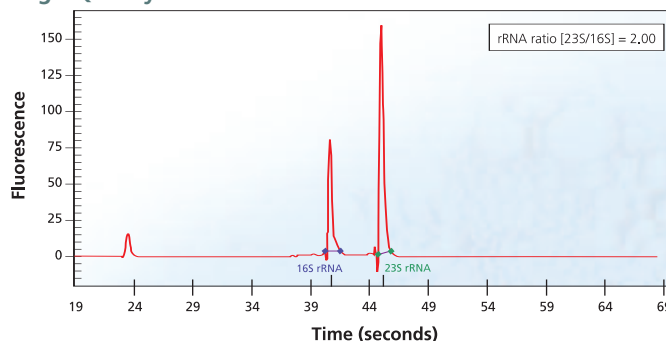
### Superior yields of RNA



**Figure 1.** Total RNA purified from *B. subtilis* using Sigma GenElute Kit and Supplier Q kit. Lanes 1, 2, 3 show increasing cell mass using Sigma kit. Lane M (center) is RNA marker (Cat. No. R7644). Lanes 4, 5, 6 show increasing cell mass using Supplier Q kit. Sigma's kit shows increasing yields with increasing cell mass whereas competitor kit achieves lower yields even with higher cell mass inputs.



### High Quality RNA



**Figure 2.** Pure intact *E. coli* RNA of high integrity is demonstrated by the Agilent 2100 Bioanalyzer electropherogram.

### Ordering Information

Cat. No.	Product Description	Preps	Quantity
<a href="#">BTR1</a>	GenElute™ Bacterial Total RNA Miniprep Kit	10	1 kit
<a href="#">BTR7</a>	GenElute™ Bacterial Total RNA Miniprep Kit	70	1 kit

# RNA Purification

## GenElute™ Yeast Total RNA Miniprep Kit

The GenElute Yeast Total RNA Miniprep Kit combines silica-membrane technology with a convenient spin column format for a rapid bind, wash, and elute method to prepare high quality total RNA from yeast.

The kit uses a yeast lysis reagent to digest cell walls and convert cells to spheroplasts. Spheroplasts are then lysed under denaturing conditions to release RNA and inactivate RNases. Addition of ethanol causes RNA to bind when the lysate is spun through a silica membrane in a microcentrifuge tube. After washing to remove contaminants, total RNA is then eluted. The purified RNA is ready for reverse transcription, PCR, labeling, microarray analysis, and other common applications.

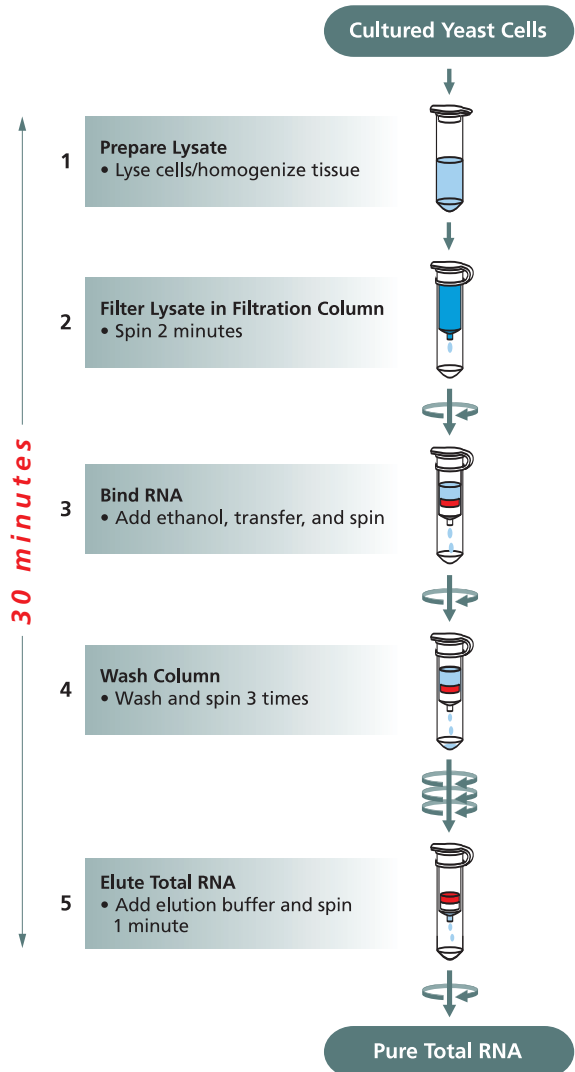
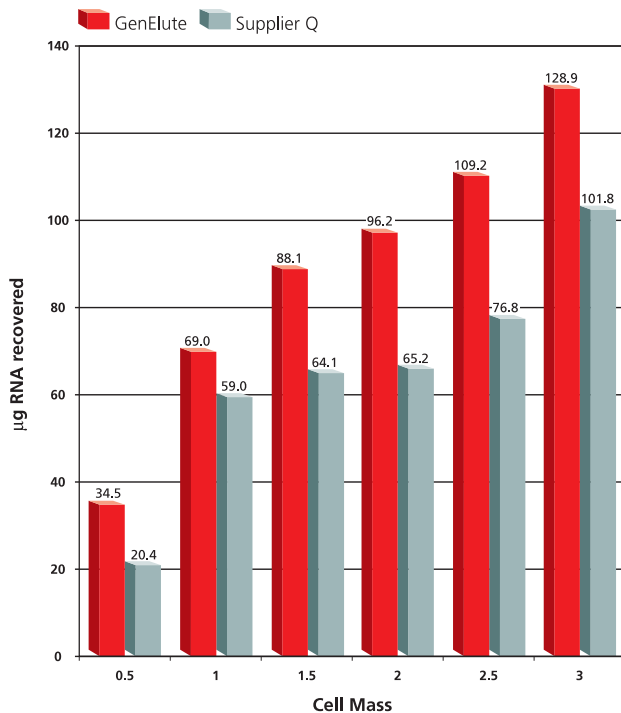
### Features and Benefits

- High Yields – up to 130 µg of pure concentrated RNA per prep
- Purity – high quality RNA in less than 1 hour
- Convenient – no cumbersome steps associated with resins and magnetic slurries

**Storage:** Room Temperature

R: 20/22-24-32-34-51/53 S: 26-36/37/39-45-60

### High yields of RNA



**Higher yields with the GenElute Yeast Total RNA Miniprep Kit.** Both Sigma and Supplier Q Total RNA kits were used as outlined in their technical bulletins for yeast RNA purification. *S. cerevisiae* was grown overnight in YPD broth at 30 °C from an isolated colony on a freshly streaked YPD plate. The culture was grown until a cell mass of 0.4-0.7 (18–24 hrs) was achieved as measured by OD<sub>600</sub>.

### Ordering Information

Cat. No.	Product Description	Preps	Quantity
<b>YTR1</b>	GenElute™ Yeast Total RNA Miniprep Kit	10	1 kit
<b>YTR7</b>	GenElute™ Yeast Total RNA Miniprep Kit	70	1 kit

# RNA Purification

## SpyLine™ Poly A+ Capture Kit

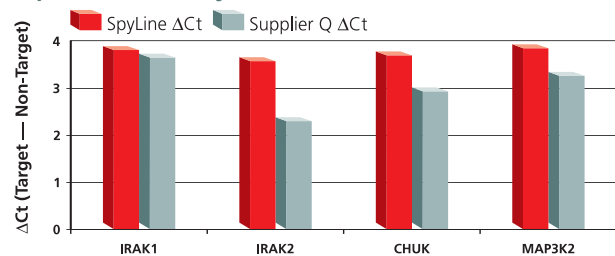
The SpyLine™ Poly A+ Capture Kit offers a simple, rapid, and cost-effective method for isolating poly A+ mRNA from cultured mammalian cells for direct RT-PCR. This kit features the SpyLine Poly A+ Plate, which is a 96-well PCR plate coated with oligo(dT) for selective capture of poly A+ mRNA from crude mammalian cell lysates. Reagents can be added directly to this capture plate for subsequent RT-PCR or real-time quantitative RT-PCR cycling and analysis. The mRNA can be eluted from the plate if desired.

### Features and Benefits

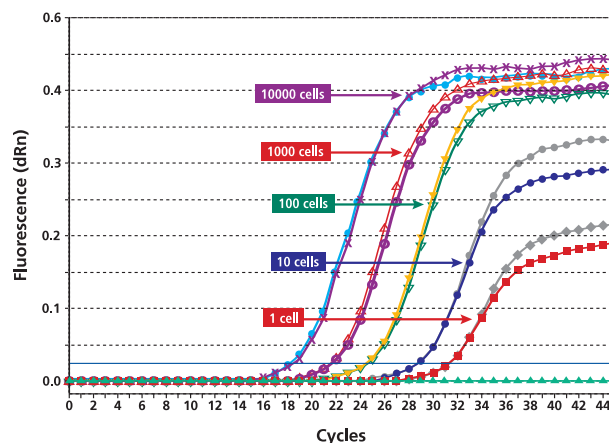
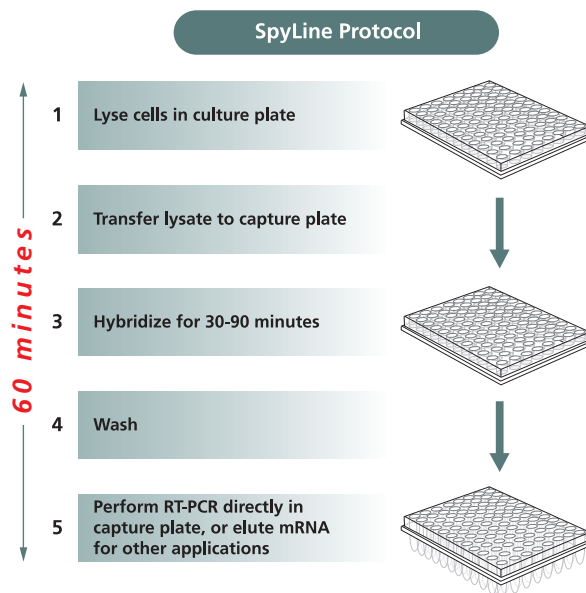
- Sensitive – superior tool for knockdown measurement and gene expression analysis
- Flexible – isolate mRNA from a wide variety of cell dilutions – even from a single cell
- Consistent – extremely low well-to-well and plate-to-plate variability
- Automatable – 96-well plate format suitable for use on high-throughput platforms
- Convenient – capture and amplify mRNA in a single plate
- Simple – rapid, easy-to-follow protocol

**Storage:** 2-8 °C

### Superior Sensitivity for siRNA Knockdown



**Figure 1. Target vs. Non-Target ΔCt for SpyLine vs. Supplier Q.** The figure above is a comparison of the change in Ct from the siRNA knockdown cells to the Non-Targeting siRNA cells. Plated HeLa cells were transfected with either siRNA transfection reagent mixture or non-targeting siRNA reagent. After overnight incubation, the transfection media was removed, and the cells were lysed. One plate was purified by the SpyLine Poly A+ Capture Kit, while the other was purified using Supplier Q's 96-well total RNA purification kit. Quantitative RT-PCR was run on the 2 sets of purified RNA using Applied Biosystems TaqMan® Gene Expression Assays.



**Figure 2. Quantitative RT-PCR of mRNA isolated from a series of decreasing cell dilutions.** Cell dilutions were prepared in replicate, in decreasing 10-fold increments from 10,000 cells to 1 cell. SURF-4 mRNAs were then amplified by quantitative RT-PCR. As depicted above, message can be clearly detected down to a single-cell input.

### Ordering Information

Cat. No.	Product Description	Plates	Quantity
<b>SPY1</b>	SpyLine™ Poly A+ Capture Kit	1 x 96	1 kit
<b>SPY4</b>	SpyLine™ Poly A+ Capture Kit	4 x 96	1 kit

# RNA Purification

## GenElute™ mRNA Miniprep Kits

*High yield isolation of mRNA from mammalian cells, tissues, or total RNA*

Procedures such as cDNA synthesis, expression profiling and others require separation of mRNA from the vastly more abundant rRNA and tRNA. The GenElute mRNA Kits provide convenient procedures for isolating polyadenylated mRNA from previously prepared total RNA or directly from mammalian cells and tissues. For direct mRNA preparation, cells or tissues are disrupted with SDS/proteinase K digestion to release RNA and eliminate RNases. Both kit types use oligo dT<sub>30</sub> covalently linked to 1 μm polystyrene beads to capture polyadenylated mRNA by hybridization. The polystyrene beads remain suspended during hybridization, eliminating the need for mixing or rocking, as is common for cellulose or magnetic particles. Polystyrene was also chosen because oligo (dT) polystyrene beads yield cleaner mRNA with fewer stringent washing steps than does the more commonly used oligo (dT) cellulose (2 or 3 wash steps versus 10 or more). With the GenElute Kits, mRNA-bead complexes are washed on a microcentrifuge spin filter, and eluted into 10 mM Tris-HCL, pH 7.5. mRNA prepared with either kit is suitable for a variety of downstream applications such as Northern blotting, Expression Array or Chip Hybridizations, and cDNA Synthesis and Library Construction.

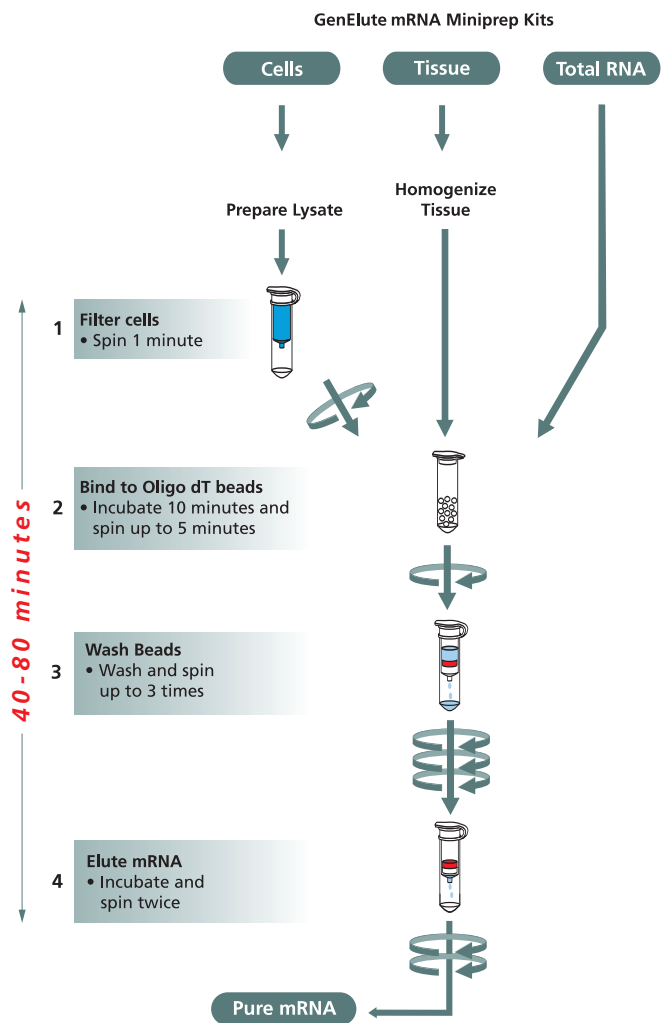
### Features and Benefits

- mRNA captured on oligo (dT) polystyrene beads in 10 minutes, with no mixing or rocking (Fig. 1)
- Poly (A)<sup>+</sup> mRNA isolated from total RNA in 40 minutes or 80 minutes directly from cells and tissues (Fig. 1)
- Oligo (dT) polystyrene beads require fewer wash steps

**Storage:** Room Temperature

MRN10, MRN70; R: 61-64-62-22-36/37 S: 53-45-36/37/39-23

DMN10, DMN70; R: 22-24-26-36/37 S: 22-24-26-36/37



### Preparation Time

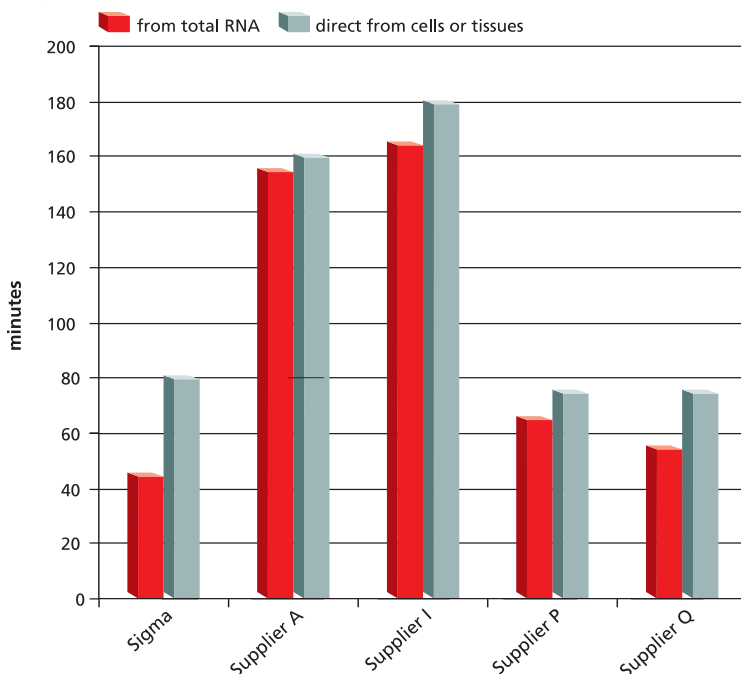
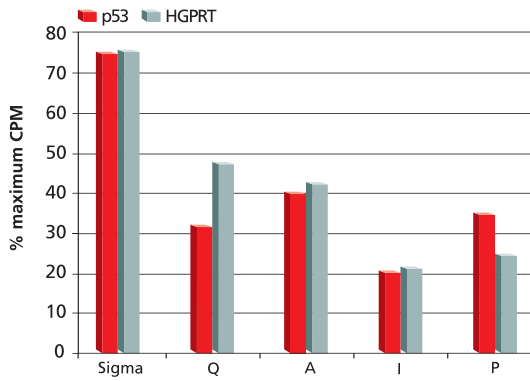


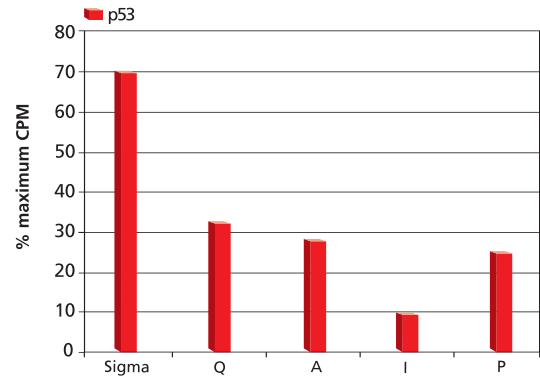
Figure 1. Comparison of the preparation time, to isolate mRNA from total RNA or direct from cells or tissues, using GenElute™ mRNA Miniprep Kit and other commercially available kits. Each kit was prepared according to the procedure supplied by the vendor.

# RNA Purification

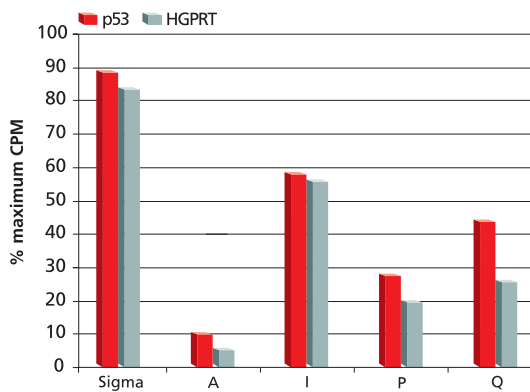
## Human mRNA Isolation from Total RNA Obtained from HEK298 Cells



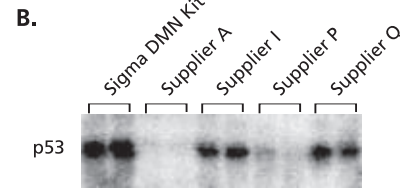
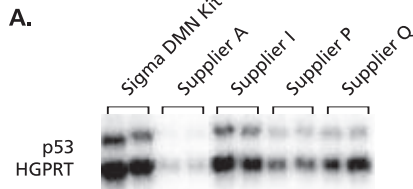
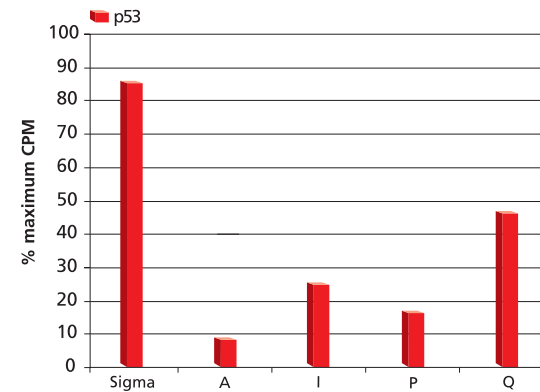
## Mouse mRNA Isolation from Total RNA Obtained from Mouse Liver



## Human mRNA Isolation Direct from HEK298 Cells



## Mouse mRNA Isolation Direct from Mouse Liver



**Figure 2.** Northern blot comparison of mRNA prepared directly from cells and tissues with GenElute Direct mRNA Miniprep Kit and competitor kits. Duplicate mRNA samples were prepared from  $5 \times 10^6$  HEK293 cells (Panel A) or 25-35 mg mouse liver (Panel B) with Sigma's GenElute Direct mRNA Miniprep Kit or with several commercially available direct mRNA miniprep kits. A portion of each mRNA preparation equal to the amount from  $1 \times 10^6$  cells or 10 mg liver was evaluated by Northern blot hybridization with  $^{32}\text{P}$ -labeled RNA probes. Hybridization was detected and quantitated by scanning the blots with a Perkin Elmer Instant Imager. Hybridization signals from each lane on the Northern blot, expressed as percent of the maximum signal for that probe, are plotted in the accompanying graphs.

## Ordering Information

Cat. No.	Product Description	Preps/Kit	Starting Material
<b>MRN10</b>	GenElute™ mRNA Miniprep Kit	10	5-500 µg total RNA
<b>MRN70</b>	GenElute™ mRNA Miniprep Kit	70	5-500 µg total RNA
<b>DMN10</b>	GenElute™ Direct mRNA Miniprep Kit	10	Up to $10^7$ mammalian cells or 50 mg tissue
<b>DMN70</b>	GenElute™ Direct mRNA Miniprep Kit	70	Up to $10^7$ mammalian cells or 50 mg tissue

# RNA Purification

## RNAlater® Storage Solution

### Tissue storage and RNA stabilization solution

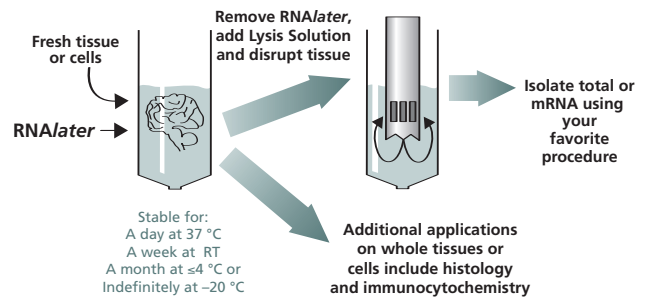
RNAlater is an aqueous, non-toxic tissue and cell storage reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNAlater eliminates the need to immediately process samples or to freeze samples in liquid nitrogen for later processing. RNAlater can be used with various downstream applications including mRNA and total RNA isolation, histology, and immunocytochemistry and is compatible with Sigma's GenElute isolation kits.

RNAlater is easy to use. Simply cut tissue samples to be stored so they are less than 0.5 cm in at least one dimension and submerge in 5 volumes of RNAlater. Small organs, such as rat kidney, liver, or spleen can be stored in whole in RNAlater. When ready to isolate the RNA, remove the tissue from RNAlater and process as though just harvested. For cell storage, resuspend pelleted cells in a small amount of PBS before adding 5-10 volumes of RNAlater. Before preparing RNA, pellet cells and discard supernatant.

### Features and Benefits

- Rapidly permeates tissues to stabilize and protect cellular RNA with immediate RNase inactivation
- Stabilizes samples at room temperature for up to one week or indefinitely at -20 °C for archiving needs
- No compromise in RNA quality following mRNA or total RNA isolation
- Aqueous non-toxic solution allows downstream tissue processing

**Storage:** Room Temperature



### Stabilizes samples at room temperature for up to one week

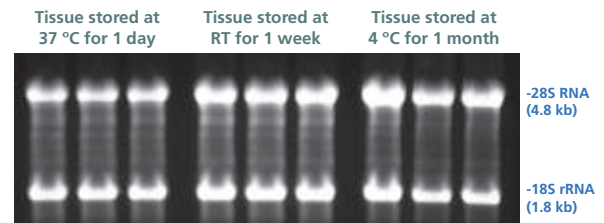
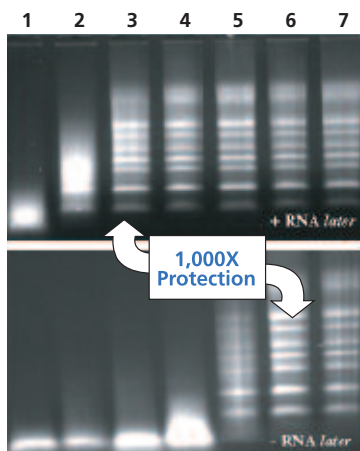


Photo courtesy of Ambion.

**Figure 1. Quality of RNA isolated from tissue stored in RNAlater Solution.** Fresh mouse tissues were dissected and stored in RNAlater at 37 °C for 1 day, room temperature for 1 week, or 4 °C for 1 month. RNA was isolated using TRI Reagent® (Cat. No. T9424) and analyzed using denaturing agarose gel electrophoresis.



### Superior protection against RNase degradation

**Figure 2.** A 5 ml aliquot of RNase A (Cat. No. R6513; serially diluted to final concentrations of  $4.5 \times 10^{-5}$  –  $4.5 \times 10^{-11}$  units/ $\mu$ l) was added to 5  $\mu$ g RNA (Cat. No. R7020) in 15  $\mu$ l containing either 10  $\mu$ l of RNAlater (top panel) or TE buffer (bottom panel). Reactions were incubated at 37 °C for 20 minutes, purified using the GenElute™ Mammalian Total RNA Miniprep Kit (Cat. No. RTN10) and analyzed on a 1% agarose gel.

## Ordering Information

Cat. No.	Product Description	Size
R0901	RNAlater® Tissue Storage and RNA Stabilization Solution	100 ml 500 ml

# RNA Purification

## Amplification Grade DNase I

### Eliminates DNA from RNA preparations

Amplification Grade DNase I (Deoxyribonuclease I) is an endonuclease isolated from bovine pancreas that digests double- and single-stranded DNA into oligo- and mono-nucleotides. DNase I is suitable for eliminating DNA from RNA preparations prior to sensitive applications, such as RT-PCR. Since no RNA purification procedure removes 100% of the DNA, RNA samples should be digested with DNase I before RT-PCR. A simple 15 minute digestion at room temperature removes the contaminating DNA. The DNase I is inactivated by adding the stop solution provided and heating. Heating also denatures the RNA, so the RNA can be used directly for reverse transcription. One kit is sufficient to treat 1,000 µg of RNA.

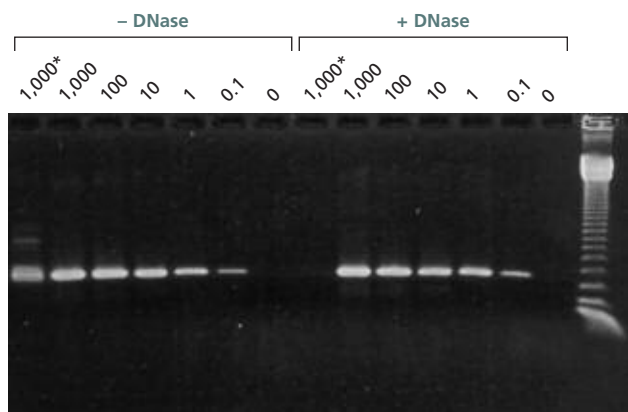
Many commercial DNase I formulations are contaminated with residual RNases. This RNase contamination can destroy or degrade valuable RNA samples prior to reverse transcription. Laboratory comparisons have shown that Sigma's Amplification Grade DNase I demonstrates lower RNase activity than that from several leading molecular biology product suppliers.

### Features and Benefits

- Suitable for the elimination of DNA from RNA preparations prior to sensitive applications such as RT-PCR
- Minimal RNase activity available
- Optimized 10x reaction buffer and Stop Solution for complete inactivation of DNase I

**Unit Definition:** One unit completely digests 1 µg of plasmid DNA to oligonucleotides in 10 min. at 37 °C.

**Storage:** -20 °C



### RT-PCR sensitivity with or without DNase digest

Figure 2. RNA was prepared from HeLa cells with the GenElute™ Mammalian Total RNA Kit. A 1,000, 100, 10, 1 or 0.1 ng aliquot of RNA was digested with Amplification Grade DNase I and amplified by RT-PCR.

\* Indicates reactions without reverse transcriptase.

**Note:** Without DNase treatment, a PCR product is obtained without reverse transcriptase, indicating that the RNA is contaminated with genomic DNA. DNase treatment eliminated this PCR-only product. RT-PCR products are visible down to 0.1 ng RNA with or without DNase, demonstrating no loss of sensitivity with the DNase treatment.

### Sigma's Amplification Grade DNase I has the lowest RNase activity

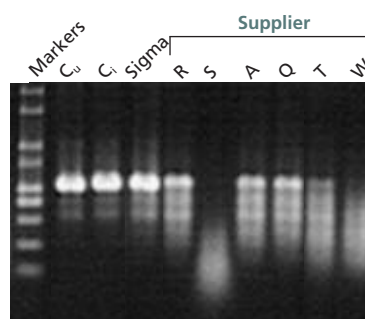


Figure 1. A 1 µg aliquot of a 1.9 kb *in vitro* transcription product was incubated with 1 unit of each DNase I at 37 °C for 1 hour and analyzed on a 1% agarose gel. C<sub>u</sub> = unincubated control (RNA in buffer without DNase, kept on ice). C<sub>i</sub> = incubated control (RNA in buffer but without DNase, incubated at 37 °C for 1 hour).

**Note:** To determine the effectiveness of DNase I treatment, parallel PCR reaction should be run without adding reverse transcriptase to check for amplification from contaminating DNA.

## Ordering Information

Cat. No.	Product Description	Quantity
AMPD1	Amplification Grade DNase I	1 kit