## GenElute™ Mammalian Total RNA Miniprep Kits

*For isolation of total RNA form mammalian cells and tissues.*

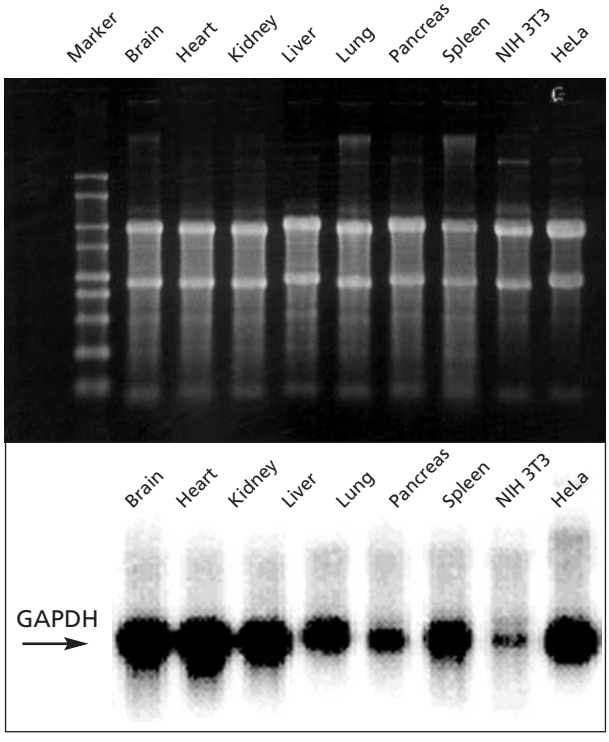
The GenElute™ Mammalian Total RNA Purification Kit combines silica-membrane technology with a convenient spin column format for a rapid bind, wash, and elute method to prepare high quality total RNA.

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 $10^7$ 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



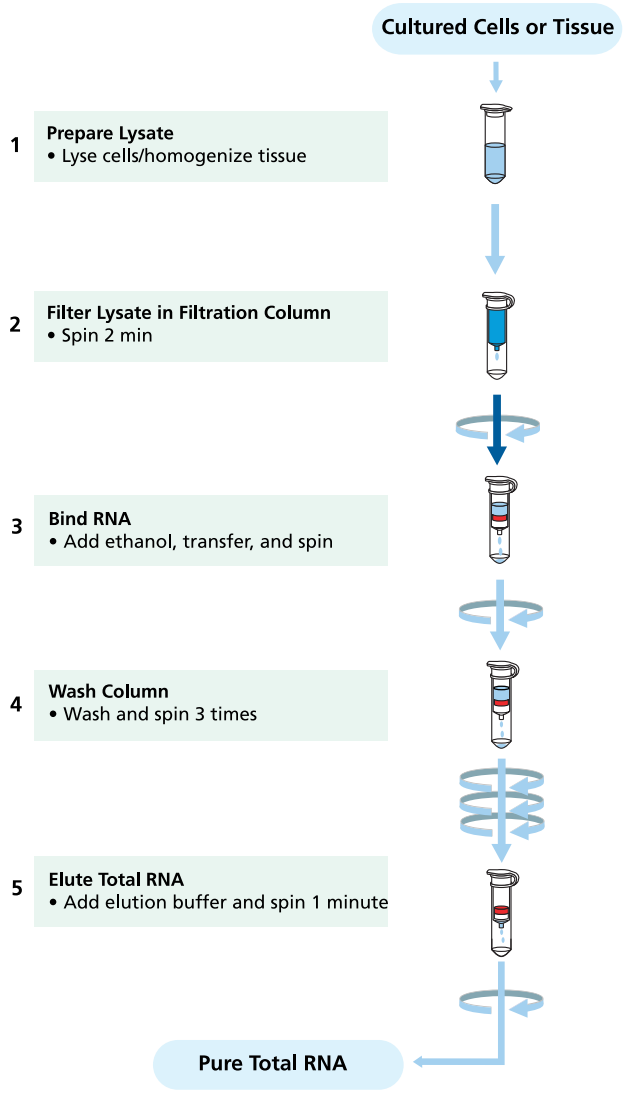

***High Quality RNA from various tissues and cells.***

***Figure 1. Formaldehyde-agarose gel and Northern blot of total RNA purified with GenElute™ Mammalian Total RNA Purification 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 $^{32}$P-labeled RNA probe for GAPDH in PerfectHyb™ Plus hybridization buffer (Product Code H 7033).*

***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

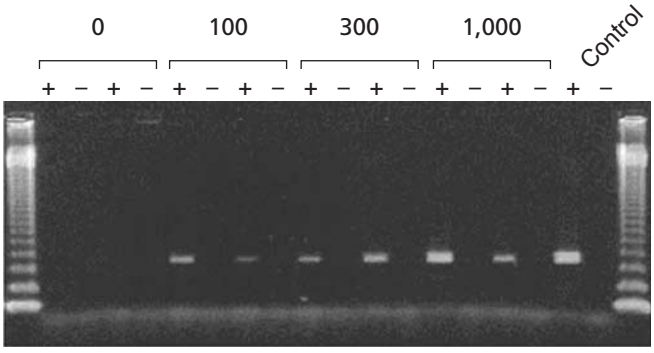



***Figure 2. RT-PCR detection with RNA from ≥ 100 cells.***

***Method:*** *HeLa cells were diluted to give 0, 100, 300, or 1,000 cells per tube, and RNA was prepared with the GenElute Mammalian Total RNA Isolation Kit. Duplicate 10 µl samples (20%) of each preparation were treated with Amplification Grade DNase I (AMP-D1). One of each pair was reverse transcribed with Enhanced Avian Reverse Transcriptase (A 4464; + lanes). The other was incubated under the same conditions, but without the reverse transcriptase (– lanes). PCR was performed with 2 µl (10%) from each reaction, G3PDH primers, and Taq DNA Polymerase (D 1806). One-fifth of each PCR product was fractionated on a 1.5% agarose gel, and photographed after staining for 30 minutes with SybrGreen I (S 9430). RT-PCR products are clearly visible for all reactions with reverse transcriptase added (+ lanes), except those with no cells (0). The control lanes contain 1 ng of Total RNA prepared form the same cell line. No PCR products are visible for reactions without reverse transcriptase added (– lanes), demonstrating that RT-PCR products with reverse transcriptase are from RNA and not from residual, contaminating DNA.*

***Comparison of total RNA recovered from Sigma's GenElute Total RNA kit and Supplier Q.***

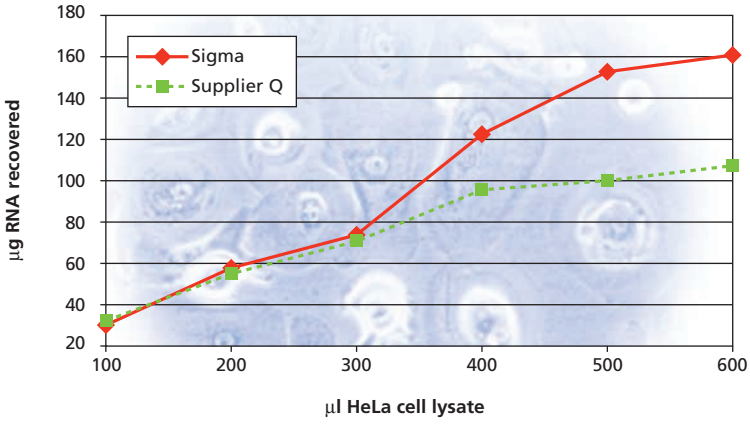



***Figure 3.*** *Total RNA was prepared from 100, 200, 300, 400, 500, and 600 µl of HeLa cell lysate (~ 1, 2, 3, 4, 5, & 6 x $10^6$ cells) with either Sigma's GenElute Mammalian Total RNA Isolation Kit or with a RNA kit from Supplier Q. The amount of RNA recovered in two 50 µl elutions was determined by absorbance at 260 nm. Note that the capacity of Supplier Q's column is ~ 100 µg, as claimed in their technical bulletin, whereas that of the Sigma column is ~ 150-160 µg. Quality of RNA prepared with the 2 kits is similar.*

## ORDERING INFORMATION

| Product | Product Description | Preps | Quantity |
|---|---|---|---|
| RTN10 | GenElute™ Mammalian Total RNA Miniprep Kit | 10 | 1 kit |
| RTN70 | GenElute™ Mammalian Total RNA Miniprep Kit | 70 | 1 kit |
| RTN350 | GenElute™ Mammalian Total RNA Miniprep Kit | 350 | 1 kit |

# RNA PURIFICATION

## TRI® Reagent RNA Isolation Reagent

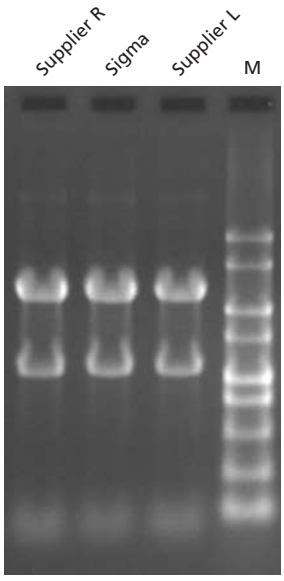
*For isolation of total RNA from a variety of starting materials.*

TRI® Reagent is an improved version of the single-step total RNA isolation reagent developed by Chomczynski.[1] The RNA isolation method based on this reagent is widely recognized and proven for RNA applications and is supported by a substantial publication list.[(2)] It is ideal for quick, economical, and efficient isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins from samples of human, animal, plant, yeast, bacterial and viral origin.

### Features and Benefits

- Easily scalable RNA isolation
- Works with many sources: human, plant, yeast, bacterial or viral
- Better yields than traditional guanidine thiocyanate/cesium chloride methods
- Three convenient formulations of TRI® Reagent

**Storage:** 4 °C
R: 23/24/25-34-31 S: 45-26-27-36/37/39




***Total RNA was prepared from HeLa cells using TRI® Reagent from Sigma and other various suppliers.***

***Figure 1.*** *Total RNA from HeLa cells was prepared using TRI® Reagents from Supplier R, Sigma and Supplier L. A 2 µl aliquot out of 200 µl total RNA was analyzed on a 1% agarose gel. RNA Marker (M) used ranged from 0.2 bp-10 kb (Product Code R 7020).*

**Table 1. TRI® Reagent Formulations**

| Product Name | Sample Type | Sample Volume | TRI® Reagent Volume |
|---|---|---|---|
| TRI® Reagent | Tissues, cultured adherent cells, cell pellets | up to 100 mg tissue, $10^7$ cells, or $10^2$ cm plate area | 1 ml |
| TRI® Reagent BD | Whole blood, plasma, serum | 0.25 ml blood derivatives | 0.75 ml |
| TRI® Reagent LS | Cell suspension, CSF, amniotic fluid | 0.25 ml fluid samples | 0.75 ml |

**Table 2. Typical RNA Yield**

| Tissue | Yield (µg RNA/mg tissue) |
|---|---|
| Liver | 6-10 |
| Spleen | 6-10 |
| Kidney | 3-4 |
| Skeletal Muscle | 1-1.5 |
| Brain | 1-1.5 |
| Placenta | 1-4 |

| Cell | Yield (µg RNA/$10^6$ cells) |
|---|---|
| Epithelial | 8-15 |
| Fibroblast | 5-7 |

## ORDERING INFORMATION

| Product | Product Description | Quantity |
|---|---|---|
| T 9424 | TRI® Reagent RNA, DNA and Protein Isolation Reagent | 25 ml<br>100 ml<br>200 ml<br>6 x 100 ml |
| T 3809 | TRI® Reagent BD | 25 ml<br>100 ml<br>200 ml |
| T 3934 | TRI® Reagent LS | 25 ml<br>100 ml<br>200 ml |
| B 9673 | 1-Bromo-3-Chloropropane (BCP) | 200 ml |


**References**

1. Chomczynski, P. and Sacchi, N., Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156 (1987).
2. Chomcznski, P. and Mackey, K., Modification of the Tri Reagent® procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *BioTechniques* **19:** 924-945 (1995).

## GenElute™ mRNA Miniprep Kits

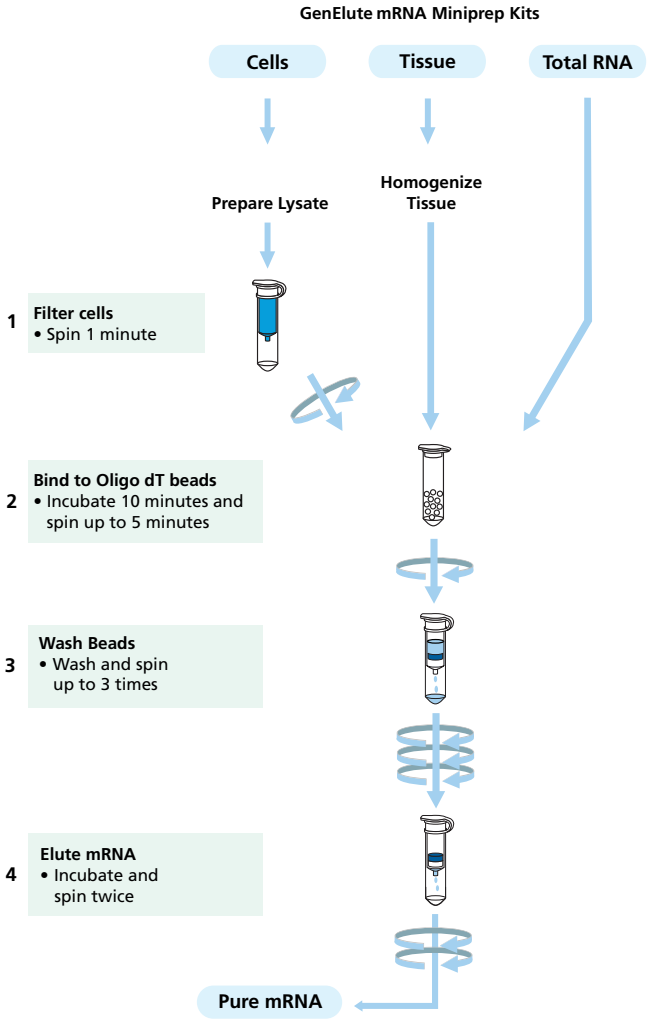
*High yield isolation of mRNA from mammalian cells, tissues, or total RNA.*

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 $dT_{30}$ 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 $A^+$ mRNA isolated from total RNA in 40 minutes (Fig. 2) or 60 minutes directly from cells and tissues (Fig. 3)
- Oligo (dt) polystyrene beads require fewer wash steps

**Storage:** Room Temperature
MRN 10, MRN 70; R: 61-64-62-22-36/37 S: 53-45-36/37/39-23
DMN 10, DMN 70; R: 20/22-42-36/37/38-41 S: 26-36-22



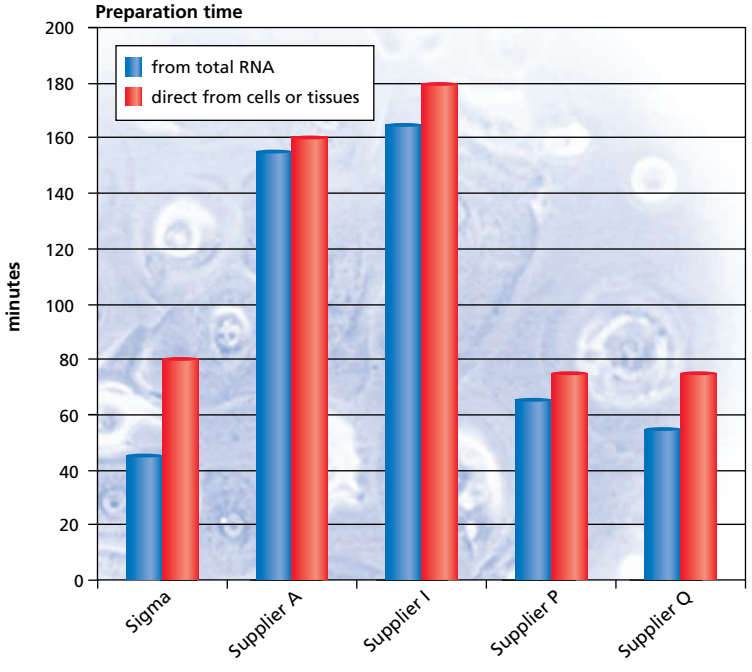




***Figure 1.*** *Comparison of the preparation time, to isolate mRNA from total RNA or direct from cells or tissues, using GenElute™ mRNA Miniprep Kit and other commercially available kits. Each kit was prepared according to the procedure supplied by the vendor.*

# RNA PURIFICATION

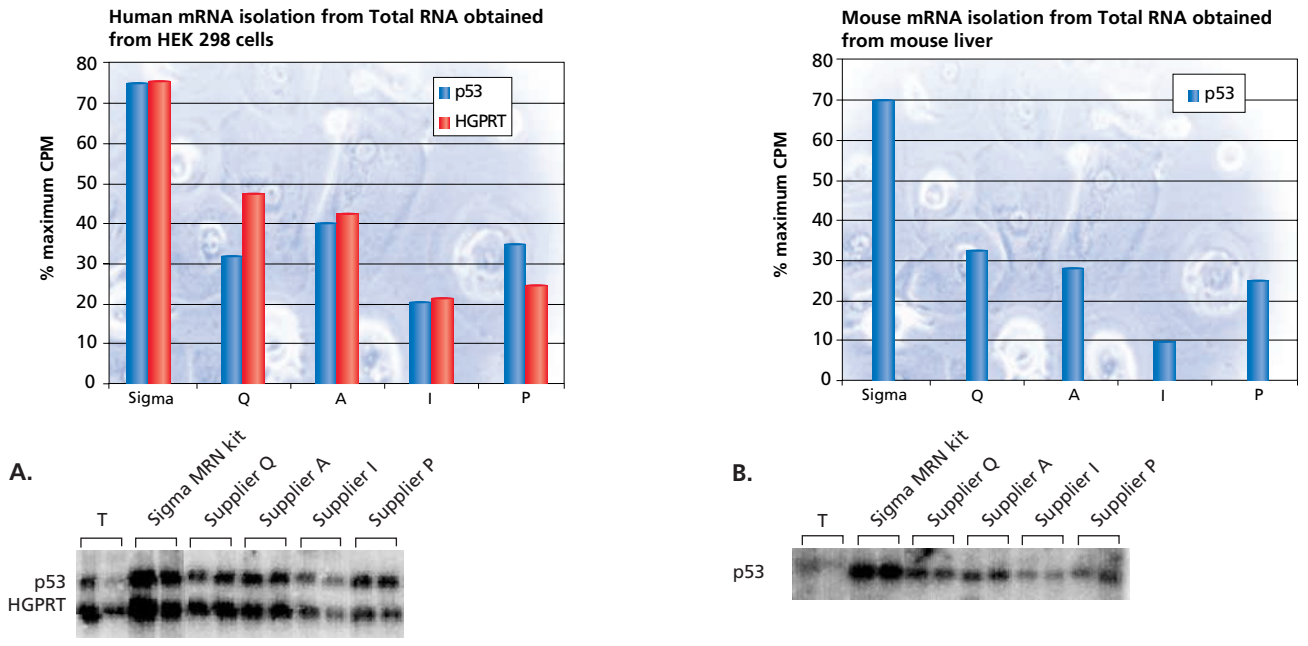



***Figure 2. Northern blot comparison of mRNA prepared from Total RNA with GenElute mRNA (MRN) & competitor kits.*** *Total RNA was prepared from Hek293 cells by a silica-binding method and from mouse liver with TriReagent. Duplicate mRNA samples were prepared from 100 µg of total RNA with Sigma's GenElute mRNA Miniprep Kit or with several commercially available mRNA miniprep kits. Twenty percent of each preparation was evaluated by Northern blot as above. In lanes* ***T,*** *5 & 2 µg of the original total RNA from cells or 10 & 5 µg of total RNA from liver were analyzed for comparison.*

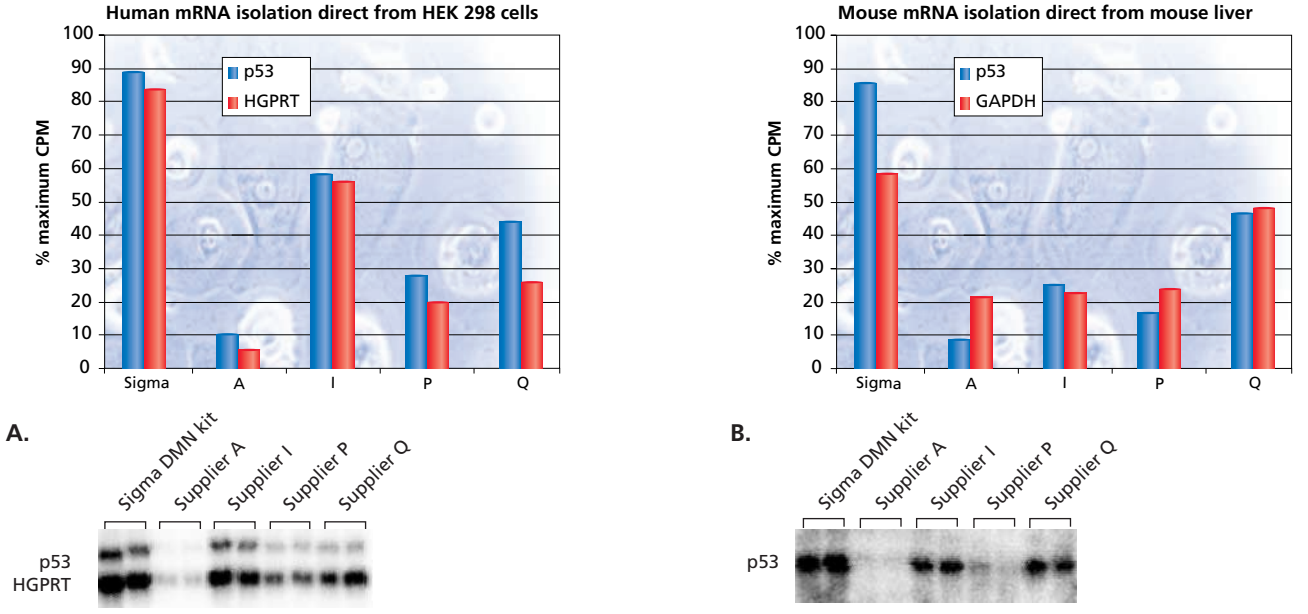



***Figure 3. Northern blot comparison of mRNA prepared directly from cells and tissues with GenElute Direct mRNA (DMN) & competitor kits.*** *Duplicate mRNA samples were prepared from 5 x $10^6$ Hek293 cells or 25-35 mg mouse liver with Sigma's GenElute Direct mRNA Miniprep Kit or with several commercially available direct mRNA miniprep kits. A portion of each mRNA preparation equal to the amount from 1 x $10^6$ cells or 10 mg liver was evaluated by Northern blot hybridization with $^{32}$P-labeled RNA probes for p53 or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA. 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

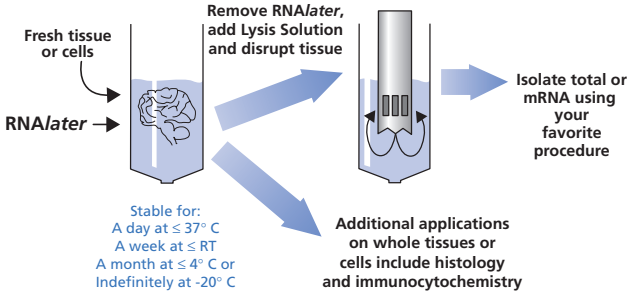
| Product | Product Description | Preps/ Kit | Starting Material |
|---|---|---|---|
| MRN 10 | GenElute™ mRNA Miniprep Kit | 10 | 5-500 µg total RNA |
| MRN 70 | GenElute™ mRNA Miniprep Kit | 70 | 5-500 µg total RNA |
| DMN 10 | GenElute™ Direct mRNA Miniprep Kit | 10 | Up to $10^7$ mammalian cells or 50 mg tissue |
| DMN 70 | GenElute™ Direct mRNA Miniprep Kit | 70 | Up to $10^7$ mammalian cells or 50 mg tissue |

## RNA*later*™ Storage Solution

### *Tissue Storage and RNA Stabilization Solution*

RNA*later* is an aqueous, non-toxic tissue and cell storage reagent that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNA*later* eliminates the need to immediately process samples or to freeze samples in liquid nitrogen for later processing. RNA*later* 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.

RNA*later* 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 RNA*later*. Small organs, such as rat kidney, liver or spleen can be stored in whole in RNA*later*. When ready to isolate the RNA, remove the tissue from RNA*later* and process as though just harvested. For cell storage, resuspend pelleted cells in a small amount of PBS before adding 5-10 volumes of RNA*later*. 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

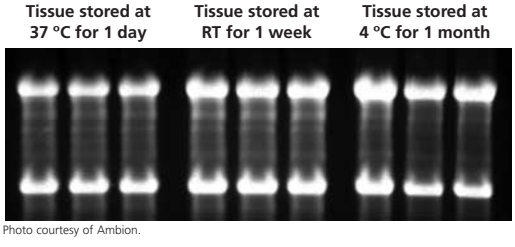
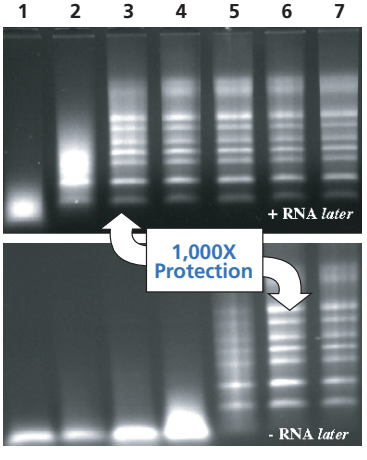



Photo courtesy of Ambion.

***Stabilizes Samples at Room Temperature For Up To One Week***

*Quality of RNA isolated from tissue stored in RNA*later *Solution. Fresh mouse tissues were dissected and stored in RNA*later *at 37 °C for 1 day, room temperature for 1 week, or 4 °C for 1 month. RNA was isolated using TRI® Reagent (MRC) and analyzed using denaturing agarose gel electrophoresis.*




***Superior Protection Against RNAse Degradation***

*A 5 ml aliquot of RNAse A (R 6513; serially diluted to final concentrations of $4.5x10^{-5}$ – $4.5x10^{-11}$ units/µl) was added to 5 µg RNA (R 7020) in 15 µl containing either 10 µl of RNA*later *(top panel) or TE buffer (bottom panel). Reactions were incubated at 37 °C for 20 minutes, purified using the GenElute™ Total RNA kit (RTN10) and analyzed on a 1% agarose gel.*

## ORDERING INFORMATION

| Product | Product Description | Size |
|---|---|---|
| R 0901 | RNA*later* Tissue Storage and RNA Stabilization Solution | 100 ml<br>500 ml |

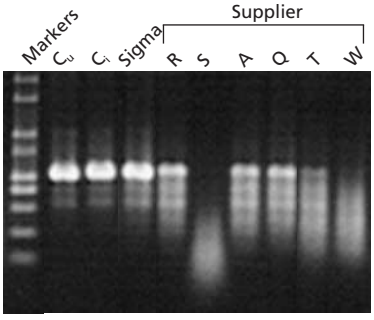
Licensed for distribution by Ambion, Inc., The RNA Company.
RNA*later*™ is a trademark of Ambion, Inc.

## 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.

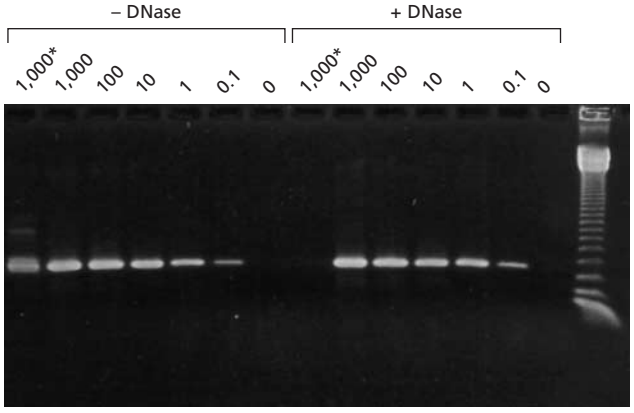
Many commercial DNase I formulations are contaminated with residual RNases. This RNase contamination can destroy or degrade valuable RNA samples prior to reverse transcription. Laboratory comparisons have shown that Sigma's Amplification Grade DNase I demonstrates lower RNase activity than that from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




***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. $C_u$ = unincubated control (RNA in buffer without DNase, kept on ice). $C_i$ = 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.*




***RT-PCR sensitivity with or without DNase digest.***

***Figure 2.*** *RNA was prepared from HeLa cells with the GenElute™ Mammalian Total RNA Kit. A 1000, 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.*

## ORDERING INFORMATION

| Product | Product Description | Quantity |
|---|---|---|
| AMP-D1 | Amplification Grade DNase I | 1 kit |