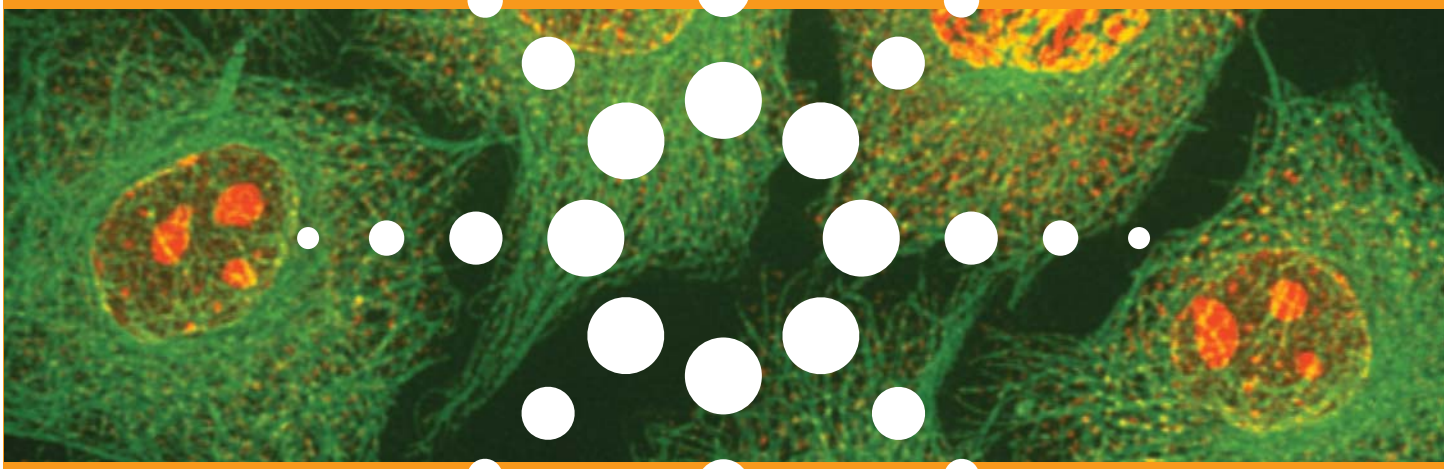
Better RNA purity.

Better data.

Without DNase treatment.



The Agilent Total RNA Isolation Kit

Agilent Technologies

Now you can isolate highly purified, intact RNA ... without DNase treatment

Introducing the Agilent Total RNA Isolation Mini Kit — The first in this line of innovative new products from Agilent

Better RNA purity and better data — in less time. That's what you get with the Total RNA Isolation Mini Kit, the latest innovation from Agilent Technologies. The kit eliminates the need for DNase treatment, with higher-purity RNA than silica-based kits using DNase treatment. And does it in less time, because there is no DNase treatment step. Highly purified, intact RNA means better data from microarray and real-time PCR experiments.

This innovative method ...

Removes up to 1,000-fold more genomic DNA contamination without DNase treatment

- Higher-purity RNA than silica-based kits with DNase treatment
- Eliminates the possibility of RNA degradation from DNase contaminated with RNase

Provides high-yield RNA — especially when sample is limited

- Elution with as little as 10µl provides concentrated RNA
- Fewer steps reduce loss of RNA

Results in improved quality and reliability of data for critical applications

- Lower background for real-time PCR improves sensitivity
- Minimizes experimental failure due to impure RNA — results in less waste of expensive reagents and microarrays

Better Purity Without DNase Treatment

The Agilent Total RNA Isolation Mini Kit is designed to remove up to 1,000 times more genomic DNA than competing silica-based kits. Agilent's unique prefiltration column significantly reduces genomic DNA contamination, eliminating the need for DNase treatment.

Total RNA was isolated from pancreas and spleen using the Agilent Total RNA Isolation Mini Kit and a silica-based kit from a leading supplier.

Genomic DNA contamination is significant in RNA isolated using a commercially available silica-based RNA isolation kit. On-column DNase digestion, at best, can reduce genomic DNA contamination to the levels obtained with the Agilent Total RNA Isolation Mini Kit without DNase-digestion. In some tissues, the level of genomic DNA contamination in RNA sample isolated with the Agilent kit is orders of magnitude lower than the silica-based kit with on-column DNase digestion.

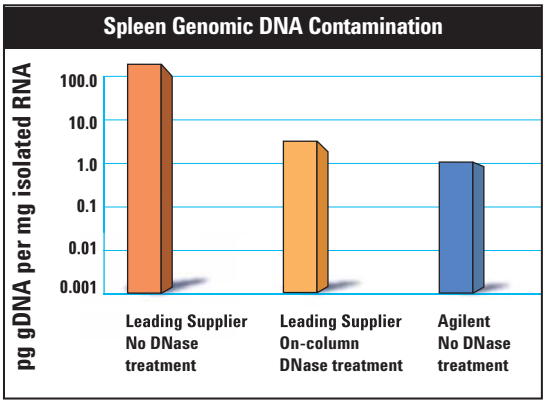


Figure 1

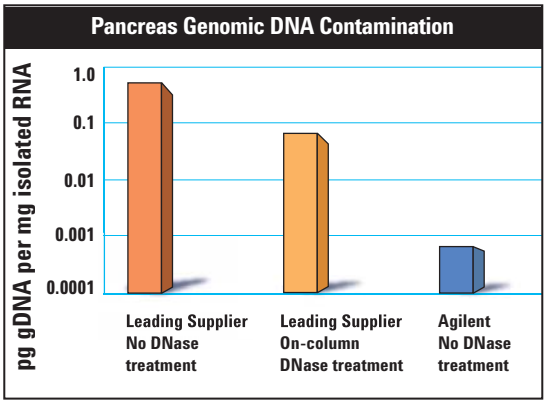


Figure 2

Figures 1 & 2:
Genomic DNA contamination was measured using real-time PCR and was performed on a real-time thermocycler using primers and probes specific to mouse GAPDH. These primers are designed to detect genomic DNA contamination if present. Typical levels of genomic DNA contamination are shown above.

High yield — especially when sample is limited

Because the DNase step is eliminated, there are fewer steps. Fewer manipulations minimize the loss of RNA, especially when sample is limited. RNA can be eluted from the isolation column in 10µl, eliminating the need for further concentration steps.

Figure 3:
Determined by optical absorbance at 260 nm, in 10 mM Tris/1 mM EDTA, pH 8 (40 µg/mL RNA per unit A260). Variation is typically 15% relative standard deviation. Recoveries for tissues are typical for a 15-mg sample.

Typical yields of cellular RNA

Sample	RNA yield
Mouse tissues	**µg/mg tissue**
Brain	0.8
Kidney	2.7
Liver	4.5
Pancreas	15
Spleen	4.7
Thymus	3.2
Cultured cells	**µg per 10^6 cells**
293HEK	10
HeLa	15
NIH3T3	15

Figure 3

Intact RNA

The quality of RNA is critical for the success of microarray-based gene expression, real-time PCR experiments, and other applications. DNase treatment is commonly used to reduce genomic DNA contamination, but problems with RNA integrity could arise if DNase is not completely RNase-free. Even extremely low RNase contamination could lead to degradation of the RNA sample during the DNase treatment. The possibility of RNA degradation is minimized when the DNase step is eliminated.

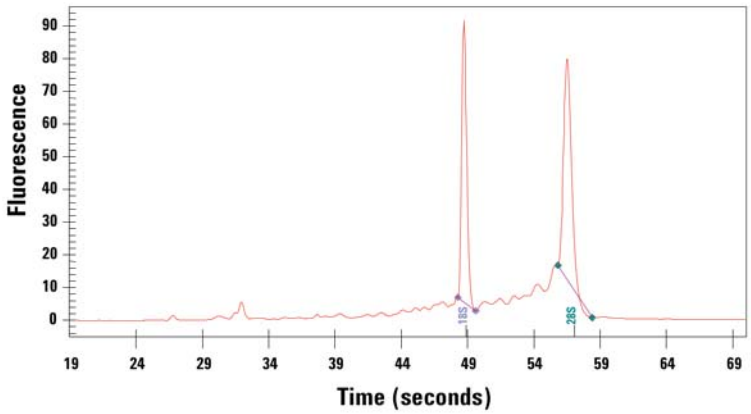


Figure 4

Figure 4:
Electropherogram and gel-like image of total RNA from mouse liver tissue obtained using Agilent 2100 bioanalyzer. Total RNA from liver was isolated using Agilent Total RNA Isolation Mini Kit. The total RNA was prepared in TE to nominal concentration 350 ng/µl and was analyzed using RNA 6000 Nano assay. This electrophoretic trace demonstrates high integrity of the total RNA in the sample.

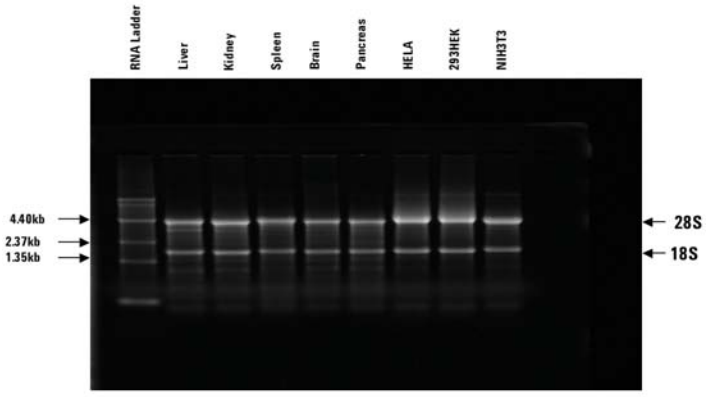


Figure 5

Figure 5:
Purity and integrity of total RNA isolated using Agilent Total RNA Isolation Mini Kit as demonstrated using Formaldehyde agarose gel. Each lane represents 1µg of total RNA from mouse tissues and cultured cells, as indicated. Clear 28s and 18s ribosomal bands and absence of high- and low-molecular smears demonstrate high purity and integrity of the total RNA in the samples.

Improved Sensitivity

A major benefit for real-time PCR users

Genomic DNA is a significant contaminant that interferes with real-time PCR and affects data reliability. This can cause problems determining true expression level of targeted messages, since PCR cannot always discriminate between cDNA targets reverse-transcribed from RNA and genomic DNA. The Agilent Total RNA Isolation Mini Kit removes significantly more genomic DNA than a commercially available silica-based kit for some tissues. When compared to this silica-based kit, RNA isolated with the Agilent kit resulted in lower background, improving sensitivity.

In Spleen

The low-cycle threshold value for the -RT sample reflects the high level of genomic DNA contamination within the RNA isolated using the silica-based kit. The Agilent-derived RNA contains 100 times less genomic DNA, as measured in the -RT sample. This reduction in genomic DNA content enables a 2-log increase in assay sensitivity.

Spleen

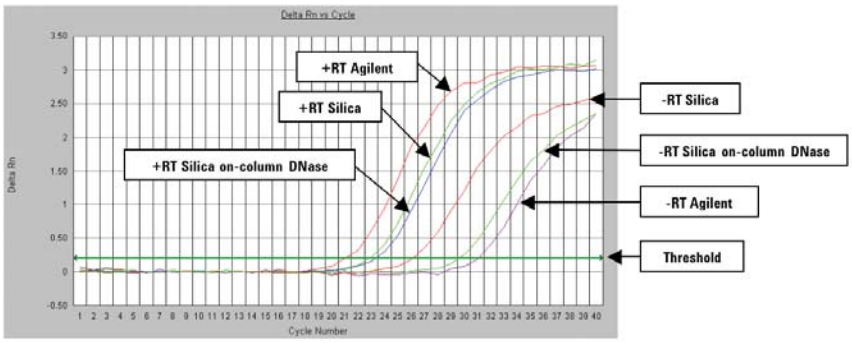


Figure 6

In Pancreas

The Agilent-derived RNA contains 1,000 times less genomic DNA than RNA isolated from the silica column, as measured in the -RT samples. This reduction in genomic DNA content enables a 3-log increase in assay sensitivity.

Pancreas

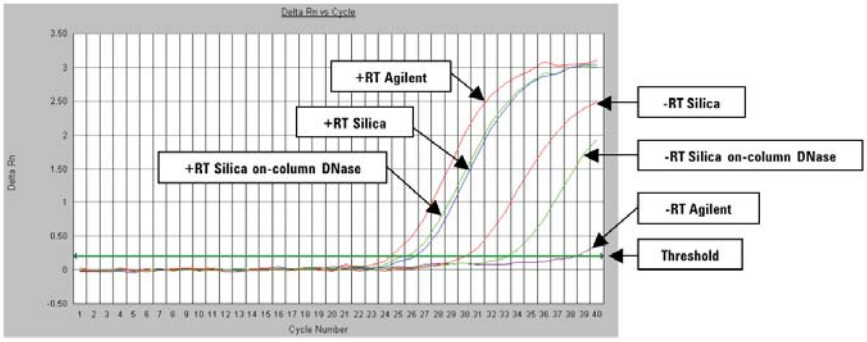


Figure 7

Figures 6 & 7:
Quantitative real-time PCR was performed on a real-time thermocycler using primer and probes specific to mouse GAPDH. Using cDNA (+RT) and negative control (-RT) templates, signal can be detected from the contaminating genomic DNA. A signal from control (-RT) samples indicates genomic DNA contamination that could contribute to the signal from cDNA (+RT).

Improved Reliability
A major benefit for microarray users

The Agilent Total RNA Isolation Mini Kit, when used to prepare RNA for microarrays, can minimize experimental failure, resulting in less waste of expensive reagents and microarrays. Reliability of gene expression experiments is assured with Agilent's Total RNA Isolation Mini Kit and Microarrays.

Agilent Isolated Spleen RNA -
Self vs Self - Mouse Developmental Oligo Array

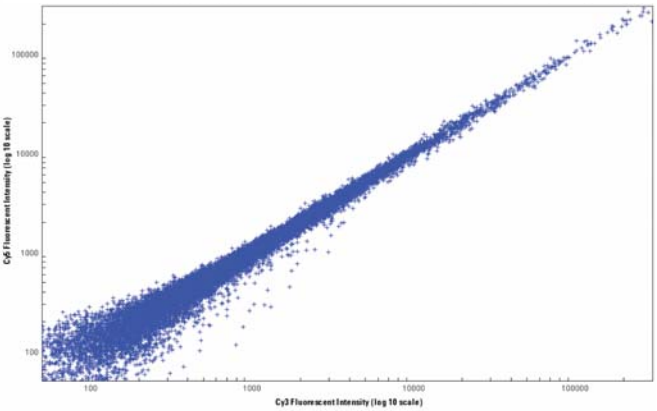


Figure 8

Figure 8:
This self vs. self hybridization shows the compatibility of all the components in Agilent's complete product solution for gene expression array analysis. RNA isolated with Agilent's RNA Isolation Kit was labeled using Agilent's Low RNA Input Fluorescent Linear Amplification Kit with Cyanine 3 and Cyanine 5, then hybridized to Agilent's Mouse Development Oligo Array. After scanning the array with Agilent's DNA Microarray Scanner System, the features are extracted and converted to multiple file formats. The extracted tiff file image is shown in this figure. The resulting fluorescent intensities of each array feature were then plotted as red vs. green, in the plot at left.

Fewer steps, no DNase treatment

Purify RNA in 20 minutes. DNase treatment step is not required, saving 15 minutes.

No special equipment required. Use a conventional roto-stator homogenizer capable of 15,000 rpm and centrifuge capable of 16,000 x g.

Prepare homogenate from mammalian tissue or cells using Lysis Solution.

Spin through prefiltration column to remove genomic DNA.

Add 70% ethanol, mix and spin, capturing RNA on isolation column.

Wash two times with Wash Solution and spin dry.

Elute pure, intact RNA with 10–50µl RNase-free water.

Kit Contents

Agilent Total RNA Isolation Mini Kit (50 purifications)

Product Number: 5185-6000

Solid phase extraction kit contains spin columns with collection tubes and reagent set sufficient for 50 isolations.

- 50 each mini prefiltration columns (color-coded natural) paired with 2 ml collection tubes
- 50 each mini isolation columns (color-coded blue) paired with 2 ml collection tubes
- 50 each 1.5 ml RNase-free final collection tubes
- Reagents Set:
  - 50 ml Lysis solution
  - 12 ml Wash solution
  - 25 ml Nuclease-free water for elution of extracted RNA

For researchers who want even better purity, Agilent will provide a **DNase Accessory Kit**. This kit will be optimized with Agilent's Total RNA Isolation Mini Kit and include highly purified DNase to minimize the possibility of RNase contamination. Look for this kit in 2004.

Format: Solid-phase mini spin columns with collection tubes

Total sample load: 5 x 10^5 - 10^6 cells, 2.5 - 30 mg tissue

Optimized for isolation of Total RNA from mammalian tissues and cells. Not intended for isolation of plant RNA or removal of unincorporated dye-labeled nucleotides prior to amplification and labeling.

The Agilent 2100 Bioanalyzer for Analysis of RNA Yield and Quality.

We recommend using the Agilent 2100 Bioanalyzer with the appropriate RNA analysis kit for assessing RNA yield and quality. This method permits analysis of RNA size, distribution, and concentration in a manner similar to gel electrophoretic methods, but with the following benefits: Minimal sample volume. Only 1µl of the RNA sample is required for analysis.
Faster analysis with higher accuracy.

- Twelve total RNA samples can be analyzed in less than one hour.
- Sample separation and image analysis are consolidated into one step, eliminating the need for separate assays.
- Integration of material handling improves the accuracy and reproducibility of the results.

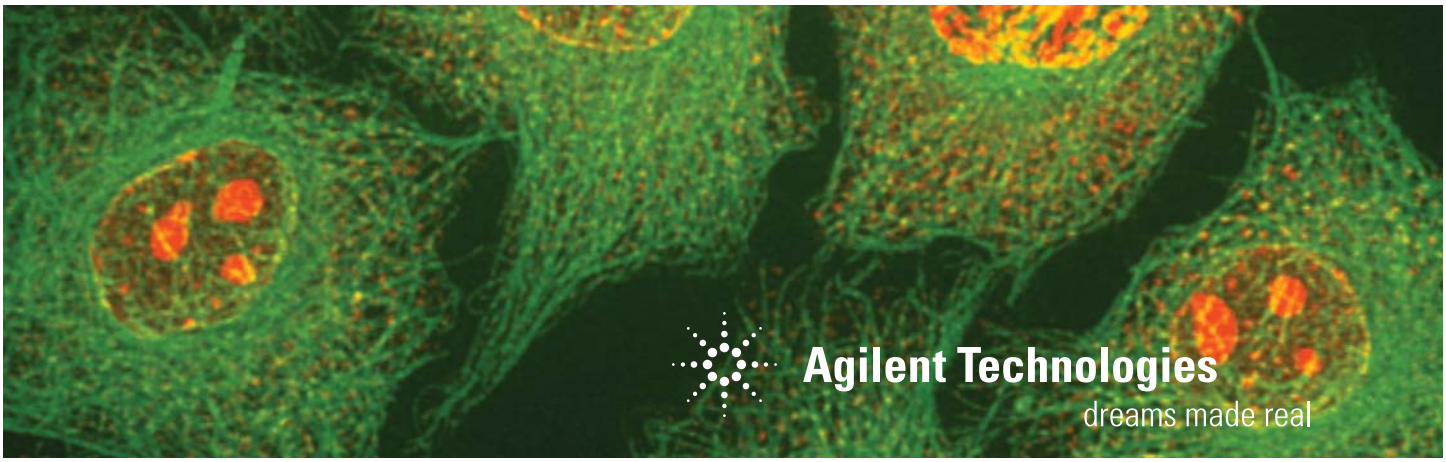
Better solutions begin with Agilent.

Agilent is dedicated to helping you maximize speed and efficiency in your laboratory. Our gene expression products, supplies and services offer **unmatched quality and reliability** for life science professionals.

You get greater efficiency and unprecedented accuracy when you use the **Agilent 2100 Bioanalyzer** to analyze RNA integrity or reverse-transcriptase PCR product.

And Agilent's **microarray solutions** put power in your hands. Highly sensitive 60-mer microarrays with Agilent SurePrint technology deliver consistent results slide-to-slide and batch-to-batch.

Look to Agilent to be your **single-source partner** — a better solution for all your gene expression needs.



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