

Systems Biology — Gene Expression Analysis

Stabilization Solutions for Reliable Gene Expression Analysis



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Why Stabilize RNA?

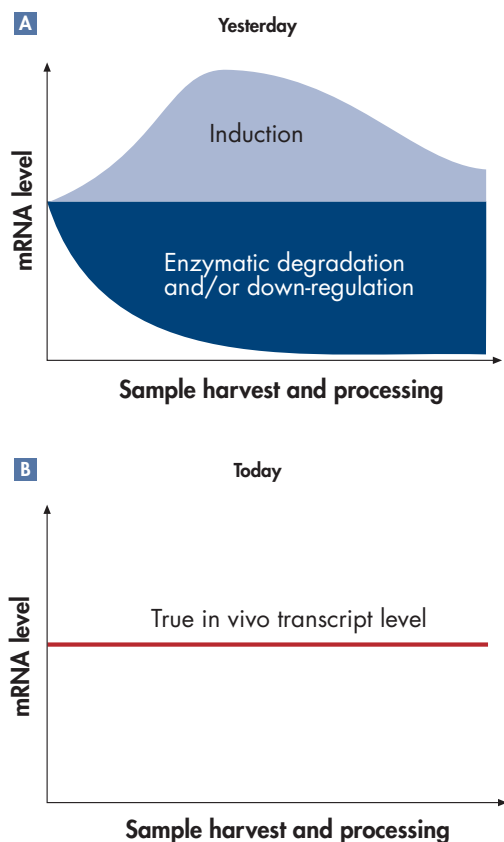


Figure 1 **A** Upon sample collection, the gene expression profile immediately begins to change. **B** Immediate RNA stabilization upon sample collection preserves the gene expression profile.

Immediate RNA stabilization

RNA in a biological sample becomes extremely unstable upon collection of the sample. QIAGEN provides products that deliver immediate RNA stabilization, preserving the gene expression profile, and enable reproducible purification of high-quality RNA. This is critical for accurate gene expression analysis in applications such as real-time RT-PCR and microarray analysis.

QIAGEN® technologies for RNA stabilization enable:

- **Immediate RNA stabilization** — for reliable gene expression analysis
- **Safe sample handling at room temperature** — no need for liquid nitrogen or dry ice
- **Convenient shipping of samples** — transportation at ambient temperature
- **Archiving of samples** — long-term storage without RNA degradation

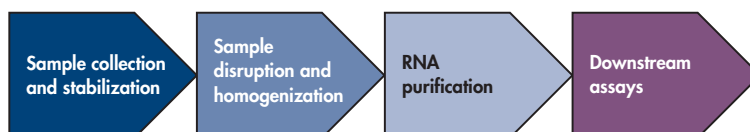
Table 1. QIAGEN Products for RNA Stabilization and Purification

Sample	RNA stabilization	RNA stabilization and purification
Tissues	RNA ^{later} ® RNA Stabilization Reagent RNA ^{later} TissueProtect Tubes	RNeasy® Protect Kits
Mammalian cells	RNAprotect® Cell Reagent	RNeasy Protect Cell Mini Kit
Human saliva	RNAprotect Saliva Reagent	RNeasy Protect Saliva Mini Kit
Human blood	PAXgene™ Blood RNA Tubes	PAXgene Blood RNA Kit*
Bacteria	RNAprotect Bacteria Reagent	RNeasy Protect Bacteria Kits

* For RNA purification only.

QIAGEN products for RNA stabilization and purification form part of QIAGEN's complete range of products that streamline and standardize the gene expression analysis workflow. For details, visit www.qiagen.com/geneXpression.

Gene Expression Analysis Workflow



Stabilization and purification of RNA from tissues

RNeasy Protect Kits enable:

- Immediate RNA stabilization in harvested tissues
- Convenient sample processing at room temperature
- Storage and shipping of tissues at 37°C (1 day), 15–25°C (7 days), or 2–8°C (4 weeks)
- Purification of high-quality RNA in minutes

The kits are supplied with RNA^{later} RNA Stabilization Reagent, which diffuses rapidly into tissues to prevent RNA degradation (Figure 2) and stabilize transcript levels (Figure 3). RNA purified using RNeasy Protect Kits is highly suited for applications such as one-step RT-PCR and microarray analysis (Figure 4 and Table 2).

RNA^{later} TissueProtect Tubes



Reclosable collection tubes containing RNA^{later} RNA Stabilization Reagent are also available separately.

Purification of Undegraded RNA

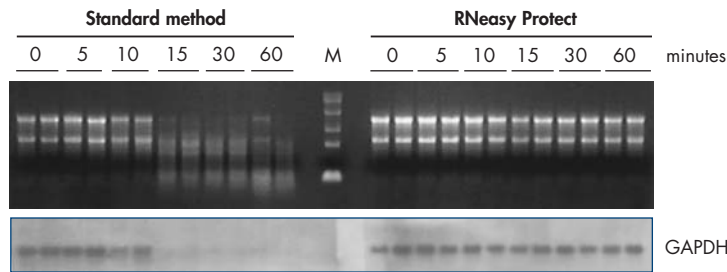


Figure 2 In contrast to a standard method, the RNeasy Protect Mini Kit allowed purification of intact RNA from rat kidney up to 60 minutes after harvesting, as shown by agarose gel and northern blot analyses. **M:** marker.

Effective Stabilization of TGF-β Transcript

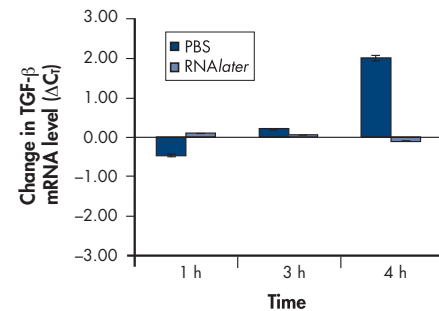


Figure 3 In contrast to PBS, RNA^{later} RNA Stabilization Reagent prevented changes in TGF-β mRNA levels in harvested rat brains for up to 4 hours. mRNA levels were quantified in triplicate by real-time RT-PCR using the QuantiTect[®] Probe RT-PCR Kit.

Reliable Analysis of the Expression of a Prostate-Specific Transgene

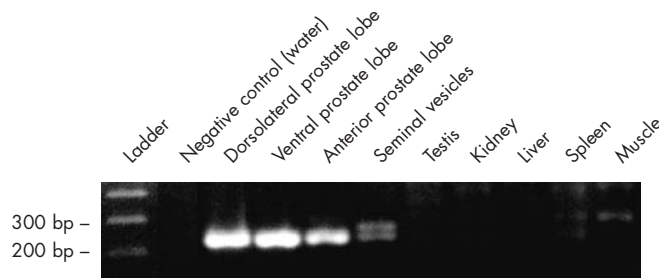


Figure 4 RT-PCR analysis of a prostate-specific transgene using RNA^{later} stabilized tissues from a transgenic mouse confirms that the transgene is present only in prostate lobes. RT-PCR was performed using the QIAGEN OneStep RT-PCR Kit.*

Table 2. Enhanced Response to Lipopolysaccharide (LPS) in 100% Sick Hemoglobin Mice

Transgenic mice with	No. genes upregulated or downregulated after LPS injection
20% sickle hemoglobin	7
100% sickle hemoglobin	413

Lung lobes from LPS-injected mice were stabilized in RNA^{later} Reagent. RNA was purified, converted to cDNA, and analyzed on a DNA microarray.¹

* Data excerpted from Kindblom, J. et al. (2003) Prostate hyperplasia in a transgenic mouse with prostate-specific expression of prolactin. *Endocrinology* **144**: 2269. Published with permission from The Endocrine Society.

¹ Data excerpted from Holtzclaw, J.D. et al. (2004) Enhanced pulmonary and systemic response to endotoxin in transgenic sickle mice. *Am. J. Respir. Crit. Care Med.* **169**: 687. Published with permission from the American Thoracic Society.

Effective Inhibition of PMA Induction of c-fos Expression

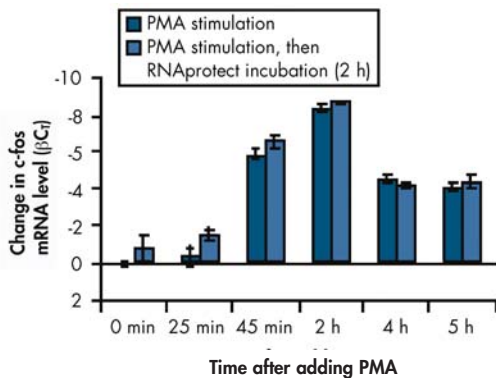


Figure 5 Incubation of PMA-stimulated Jurkat cells in RNAprotect Cell Reagent prevents PMA induction of c-fos expression as well as degradation of c-fos transcript, demonstrating stabilization of RNA by RNAprotect Cell Reagent (each bar represents the mean of duplicate cell samples).

Table 3. Higher RNA Yields from Preserved Leukocytes

	Preservation for 10 days in RNAprotect Cell Reagent	RNA later Reagent
RNA yield using RNeasy technology (μg)	10	3.5
Real-time RT-PCR of abl (C _T value)	25	26

Leukocytes were isolated on a Ficoll® gradient.

Data kindly provided by Pascale Cornillet-Lefebvre, Hôpital Robert Debré, Reims, France.

Table 4. Intact RNA from Jurkat Cells Stored at Ambient Temperature

Cell storage conditions	RIN value of purified RNA
30°C (1 day)	9.8
25°C (7 days)	9.7
2–8°C (28 days)	10
–20°C (28 days)	9.8
–80°C (28 days)	9.8

RNA integrity is assessed on the Agilent® 2100 bioanalyzer, which gives RIN values on a scale of 1 (completely degraded RNA) to 10 (completely intact RNA).

Stabilization and purification of RNA from cells

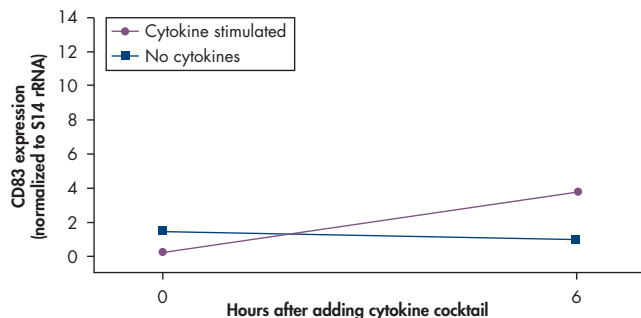
Benefits of the RNeasy Protect Cell Mini Kit:

- Immediate RNA stabilization in sorted or cultured cells with no need to remove medium
- Storage and shipping of cells at 30°C (1 day), 15–25°C (7 days), or 2–8°C (4 weeks), instead of on dry ice
- Archiving of cells at –20°C or –80°C
- Purification of high-quality RNA in minutes

The kit contains RNAprotect Cell Reagent, which effectively stabilizes transcript levels (Figures 5 and 6 and Table 3) and keeps RNA intact (Table 4). The purified RNA gives reproducible results in applications such as real-time RT-PCR.

More Reliable Quantification of CD83 Transcript

Cells stored in PBS



Cells stored in RNAprotect Cell Reagent

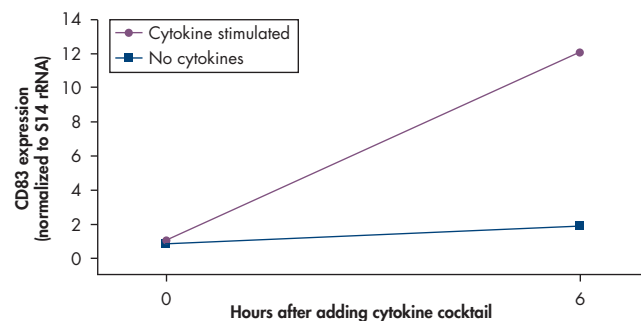


Figure 6 Storage of cytokine-stimulated immature dendritic cells overnight at –20°C in RNAprotect Cell Reagent instead of PBS allows quantification of higher levels of CD83 mRNA, indicating stabilization of RNA by RNAprotect Cell Reagent. Data kindly provided by Dr. Alexander Prechtel and Prof. Alexander Steinkasserer, University Hospital Erlangen, Erlangen, Germany.

Stabilization and purification of RNA from human saliva

The RNeasy Protect Saliva Mini Kit provides:

- Immediate RNA stabilization in saliva samples at room temperature
- Storage and shipping of saliva samples at 37°C (1 day), 15–25°C (14 days), or 2–8°C (4 weeks)
- Archiving of saliva samples at –20°C or –80°C
- Purification of high-quality RNA in minutes

RNAprotect Saliva Reagent, supplied in the kit, effectively stabilizes transcript levels (Figures 7 and 8). Gene expression analysis of saliva represents a potential, noninvasive alternative to analysis of blood (Figure 9). The kit allows research in the identification of possible RNA biomarkers for oral and systemic diseases.

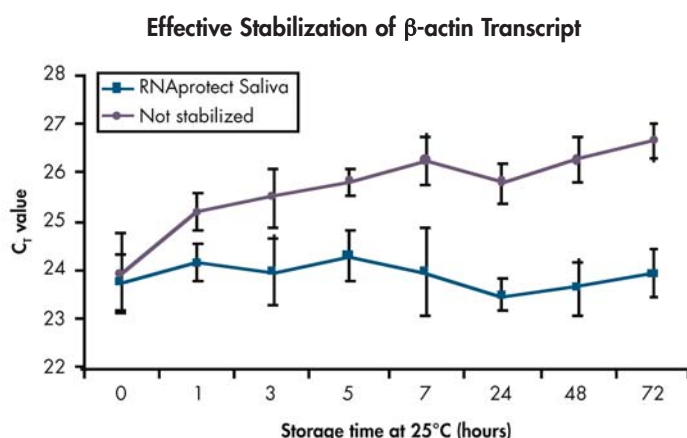
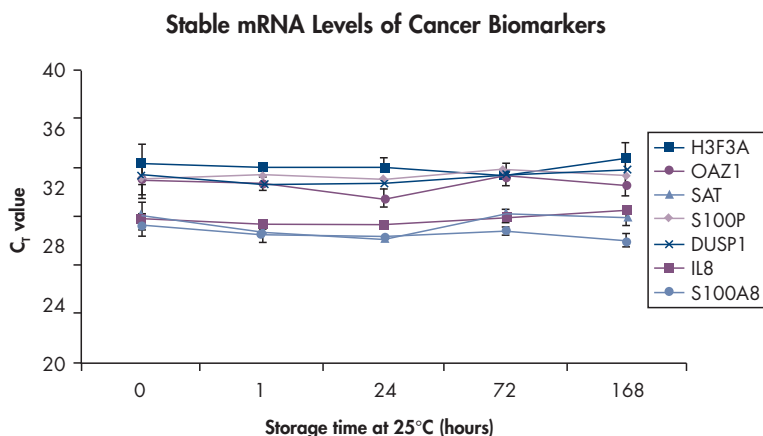


Figure 7 RNAprotect Saliva Reagent stabilized β -actin mRNA levels in saliva for up to 3 days at 25°C. mRNA levels were quantified by real-time RT-PCR using the QuantiTect Probe RT-PCR Kit. Each time point shows the mean C_t value from 4 or 6 samples from a saliva pool from 3 donors.



Minimization of Transcript Degradation in Saliva

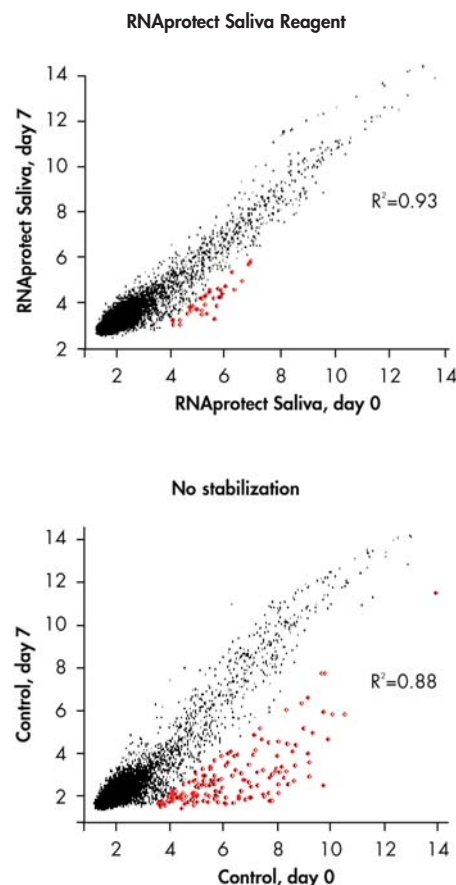


Figure 8 Saliva samples were incubated with or without RNAprotect Saliva Reagent at room temperature for 0 or 7 days. After RNA purification, samples were analyzed using Human Genome U133 Plus 2.0 GeneChip® Arrays. Comparison between 0 and 7 days shows a 2-fold decrease in signal intensity for 159 transcripts with unstabilized samples, but for only 38 transcripts with RNAprotect stabilized samples (indicated by red spots). Data kindly provided by Dr. David Wong, University of California, Los Angeles, CA, USA.

Figure 9 RNAprotect Saliva Reagent stabilized mRNA levels of oral cancer biomarkers for up to 168 hours. Each time point shows the mean C_t value from real-time RT-PCR analysis of 3 samples from a saliva pool from 9 donors. **H3F3A**: H3 histone, family 3A; **OAZ1**: ornithine decarboxylase antizyme 1; **SAT**: spermidine/spermine N1-acetyltransferase; **S100P**: S100 calcium binding protein P; **DUSP1**: dual specificity phosphatase 1; **IL8**: interleukin 8; **S100A8**: S100 calcium binding protein A8 (an abundant mRNA in saliva, not a cancer biomarker). Data kindly provided by Dr. David Wong, University of California, Los Angeles, CA, USA.

Comparison of Two *E. coli* Lineages

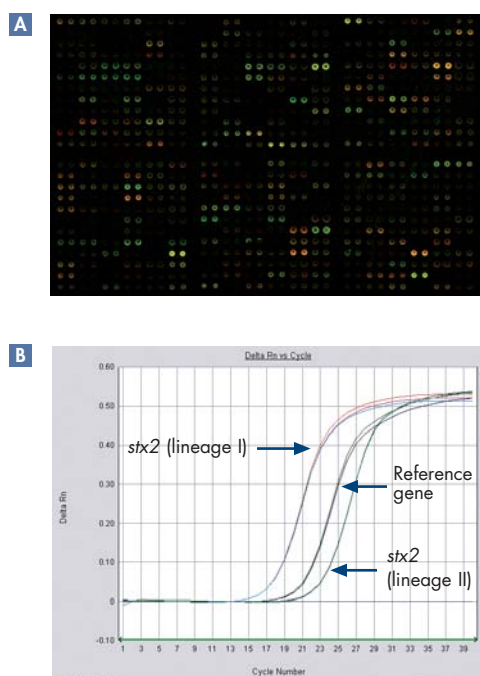


Figure 10 RNA purified from two lineages of *E. coli* O157:H7 with the RNeasy Protect Bacteria Mini Kit was used in **A** microarray analysis of virulence related genes (RNA from one lineage was Cy³ labeled, RNA from the other was Cy5 labeled) and **B** real-time RT-PCR analysis of *stx2*. Data kindly provided by Dr. Scot Dowd, Livestock Issues Research Unit, Lubbock, TX, USA.

Stabilization and purification of RNA from human blood

Benefits of the PAXgene Blood RNA System:

- Integrated system for collection, stabilization, and purification of intracellular RNA
- RNA stabilization for up to 3 days at 18–25°C
- Standardized sample processing prior to analysis

The PAXgene Blood RNA System is for in vitro diagnostic use in the USA and the European Union. The system comprises PAXgene Blood RNA Tubes for the collection, stabilization, and transport of blood and the PAXgene Blood RNA Kit for subsequent RNA purification (Figure 11).

Stabilization and purification of RNA from bacteria

RNeasy Protect Bacteria Kits allow:

- In vivo stabilization of RNA in bacterial cultures
- Safe storage of stabilized bacteria at –20°C (2 weeks) or –70°C (4 weeks)
- Purification of high-quality RNA in minutes

RNeasy Protect Bacteria Kits enable the stabilization and purification of RNA from Gram-positive and Gram-negative bacteria. The purified RNA is suitable for applications such as microarray analysis and real-time RT-PCR (Figure 10).

PAXgene System Stabilizes c-fos mRNA Levels for up to 3 Days

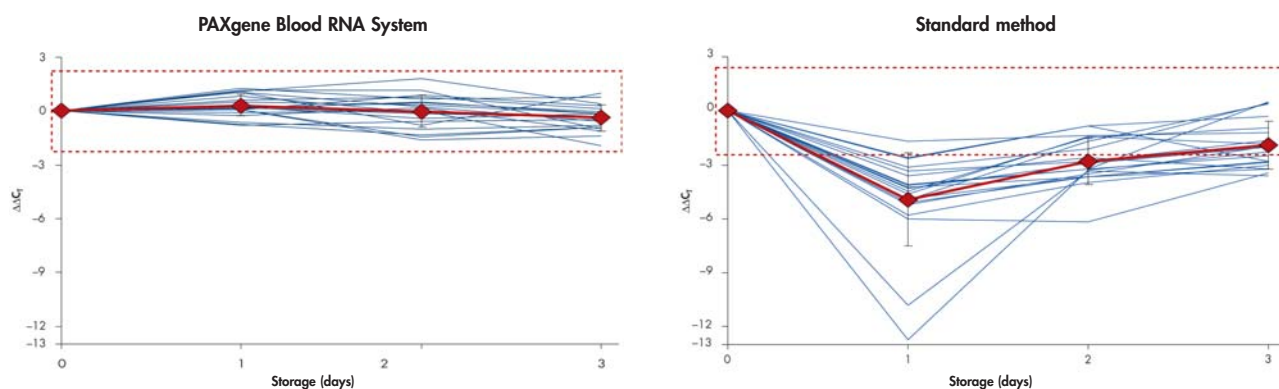


Figure 11 c-fos mRNA levels in human blood remain stable at 18–25°C for 1–3 days when blood is collected and RNA is purified using the PAXgene Blood RNA System instead of a standard method. Duplicate blood samples from 10 donors were analyzed; dashed lines indicate the $\pm 3x$ total precision of the real-time RT-PCR assay (2.34 C_T).

Ordering Information

Product	Contents	Cat. no.
Products for tissues		
RNA ^{later} RNA Stabilization Reagent (50 ml)	For stabilization of RNA in 25 x 200 mg tissue samples: 50 ml RNA ^{later} RNA Stabilization Reagent	76104
RNA ^{later} RNA Stabilization Reagent (250 ml)	For stabilization of RNA in 125 x 200 mg tissue samples: 250 ml RNA ^{later} RNA Stabilization Reagent	76106
RNA ^{later} TissueProtect Tubes (50 x 1.5 ml)	For stabilization of RNA in 50 x 150 mg tissue samples: 50 screw-top tubes containing 1.5 ml RNA ^{later} RNA Stabilization Reagent each	76154
RNA ^{later} TissueProtect Tubes (20 x 5 ml)	For stabilization of RNA in 20 x 500 mg tissue samples: 20 screw-top tubes containing 5 ml RNA ^{later} RNA Stabilization Reagent each	76163
RNeasy Protect Mini Kit (50)*	RNA ^{later} RNA Stabilization Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes, RNase-Free Reagents and Buffers	74124
Products for mammalian cells		
RNAprotect Cell Reagent (250 ml)	250 ml RNAprotect Cell Reagent	76526
RNeasy Protect Cell Mini Kit (50)	RNAprotect Cell Reagent (50 ml) and RNeasy Plus Mini Kit (50)	74624
Product for human saliva		
RNeasy Protect Saliva Mini Kit (50)	RNAprotect Saliva Reagent (50 ml) and RNeasy Micro Kit (50)	74324
Product for human blood		
PAXgene Blood RNA Kit (50)	50 PAXgene Spin Columns, 50 PAXgene Shredder Spin Columns, Processing Tubes, RNase-Free DNase I, RNase-Free Reagents and Buffers. To be used in conjunction with PAXgene Blood RNA Tubes [†]	762164 [†] 762174 [§]
Products for bacteria		
RNAprotect Bacteria Reagent	RNAprotect Bacteria Reagent (2 x 100 ml)	76506
RNeasy Protect Bacteria Mini Kit (50)	RNAprotect Bacteria Reagent (2 x 100 ml) and RNeasy Mini Kit (50)	74524
RNeasy Protect Bacteria Midi Kit (10)	RNAprotect Bacteria Reagent (2 x 100 ml) and RNeasy Midi Kit (10)	75552

* Larger kit size available; please inquire.

[†] PAXgene Blood RNA Tubes are available from BD and BD authorized distributors (www.bd.com).

[‡] Canada and USA only. [§] Rest of the world; kit not available in all countries.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

"RNA^{later}" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

Trademarks: QIAGEN®, QuantiTect®, RNAprotect®, RNeasy® (QIAGEN Group); Agilent® (Agilent Technologies, Inc.); Cy®, Ficoll® (General Electric Company); PAXgene™ (PreAnalytiX GmbH).

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Gene Expression Analysis

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China = Orders 021-51345678 = Fax 021-51342500 = Technical 021-51345678

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