# RT² Profiler PCR Arrays: Pathway Analysis

**The complete PCR array technical reference**

Pathway and disease research profiling

- Apoptosis
- Biomarkers
- Cancer research
- Cell Cycle
- Cytokines
- ECM/adhesion
- Neuroscience
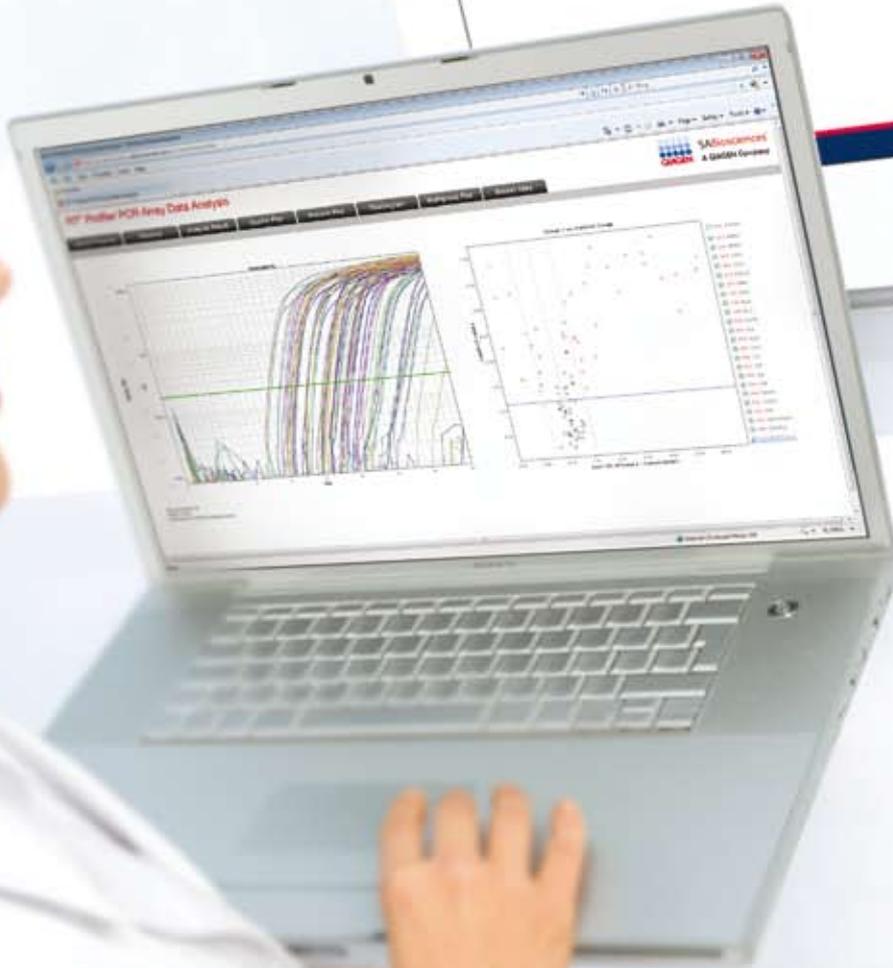- Signal transduction
- Stem cell/development
- Toxicology/drug ADME

**QIAGEN**®

**Sample & Assay Technologies**

# Table of contents

| Compatible instruments | | Plate |
|---|---|---|
| **QIAGEN** | Rotor-Gene® Q, Rotor-Gene 6000 | R |
| **Applied Biosystems (ABI)** | ABI 5700, 7000, 7300 | A |
| | ABI 7500 Standard, ViiA™ 7 (96-well block) | A |
| | ABI 7500 FAST, ViiA 7 FAST (96-well Block) | C |
| | ABI 7900HT Standard (96-well Block), 7700 | A |
| | ABI 7900HT FAST (96-well Block) | C |
| | ABI 7900HT, ViiA 7 (384-well Block) | E |
| | ABI StepOnePlus™ | C |
| **Bio-Rad** | iCycler®, iQ™5, MyiQ™, MyiQ2, Chromo4™ | A |
| | CFX96™, Opticon® 2 | D |
| | CFX384™ | E |
| **Stratagene** | Mx3000P®, Mx3005P® | A |
| | Mx4000® | D |
| **Roche** | LightCycler® 480 (96-well Block) | F |
| | LightCycler 480 (384-well Block) | G |
| **Eppendorf** | Mastercycler® ep realplex 2/2S, 4/4S | A |
| **TaKaRa** | TP-800 | A |
| **Fluidigm** | BioMark™ | H |

| RT² Profiler PCR Array accessory | | Cat. no. |
|---|---|---|
| **RT² First Strand Kit** | 12 samples | 330401 |

| Master Mix for RT² Profiler PCR Arrays | | Cat. no. |
|---|---|---|
| **RT² SYBR® Green** | 2 arrays (96-well) | 330520 |
| **ROX™ qPCR Mastermix** | 12 arrays (96-well) | 330522 |
| | 24 arrays (96-well) | 330523 |
| | 4 arrays (384-well) | 330521 |
| **RT² SYBR Green Fluor qPCR Mastermix** | 2 arrays (96-well) | 330510 |
| | 12 arrays (96-well) | 330512 |
| | 24 arrays (96-well) | 330513 |
| | 4 arrays (384-well) | 330511 |
| **RT² SYBR Green qPCR Mastermix** | 2 arrays (96-well) | 330500 |
| | 12 arrays (96-well) | 330502 |
| | 24 arrays (96-well) | 330503 |
| | 4 arrays (384-well) | 330501 |

| Popular RT² Profiler PCR Arrays for research | Cat. no.* SAP # / PCR Array # |
|---|---|
| Angiogenesis | 330231 / PAXX-024Y |
| Apoptosis | 330231 / PAXX-012Y |
| Autophagy | 330231 / PAXX-084Y |
| Breast Cancer and Estrogen Receptor Signaling | 330231 / PAXX-005Y |
| Cancer PathwayFinder | 330231 / PAXX-033Y |
| Cell Cycle | 330231 / PAXX-020Y |
| Chemokines and Receptors | 330231 / PAXX-022Y |
| Diabetes | 330231 / PAXX-023Y |
| DNA Damage Signaling Pathway | 330231 / PAXX-029Y |
| Drug Metabolism | 330231 / PAXX-002Y |
| EGF/PDGF Signaling Pathway | 330231 / PAXX-040Y |
| Embryonic Stem Cells | 330231 / PAXX-081Y |
| Endothelial Cell Biology | 330231 / PAXX-015Y |
| Epigenetic Chromatin Modification Enzymes | 330231 / PAXX-085Y |
| Epithelial to Mesenchymal Transition | 330231 / PAXX-090Y |
| Extracellular Matrix and Adhesion Molecules | 330231 / PAXX-013Y |
| GPCR Signaling Pathway | 330231 / PAXX-071Y |
| Growth Factors | 330231 / PAXX-041Y |
| Heat Shock Proteins | 330231 / PAXX-076Y |
| Hedgehog Signaling Pathway | 330231 / PAXX-078Y |
| Hematopoietic Stem Cells & Hematopoesis | 330231 / PAXX-054Y |
| Hepatoxicity | 330231 / PAXX-093Y |
| Hypoxia Signaling Pathway | 330231 / PAXX-032Y |
| Inflammatory Cytokines and Receptors | 330231 / PAXX-011Y |
| Innate and Adaptive Immune Response | 330231 / PAXX-052Y |
| Interferon $\alpha,\beta$ Response | 330231 / PAXX-016Y |
| JAK/STAT Signaling Pathway | 330231 / PAXX-039Y |
| MAP Kinase Signaling Pathway | 330231 / PAXX-061Y |
| Mitochondrial Energy Metabolism | 330231 / PAXX-008Y |
| Nephrotoxicity | 330231 / PAXX-094Y |
| NFκB Signaling Pathway | 330231 / PAXX-025Y |
| Oxidative Stress and Antioxidant Defense | 330231 / PAXX-065Y |
| p53 Signaling Pathway | 330231 / PAXX-027Y |
| Wnt Signaling Pathway | 330231 / PAXX-043Y |

* XX = species; Y = plate format.

# What are RT² Profiler PCR Arrays?

RT² Profiler PCR Arrays are a highly reliable and sensitive gene expression profiling technology for analyzing focused panels of genes in signal transduction, biological processes, or disease research pathways using real-time PCR.

Each cataloged RT² Profiler PCR Array contains a list of the pathway-focused genes as well as 5 housekeeping (reference) genes on the array. In addition, each array contains a panel of proprietary controls to monitor genomic DNA contamination (GDC) as well as the first strand synthesis (RTC) and real-time PCR efficiency (PPC).

## Why use RT² Profiler PCR Arrays?

- **Simplicity:** The simplicity of RT² Profiler PCR Arrays makes routine expression profiling practical in any research laboratory with a real-time instrument.

- **Performance:** RT² Profiler PCR Arrays have the sensitivity, reproducibility, specificity, and reliability to accurately profile multiple genes simultaneously in 96- and 384-well plate, 100-well disc, and 96x96 chip formats.

- **Relevance:** RT² Profiler PCR Arrays focus on profiling the genes relevant to the pathways or disease states important to your research.
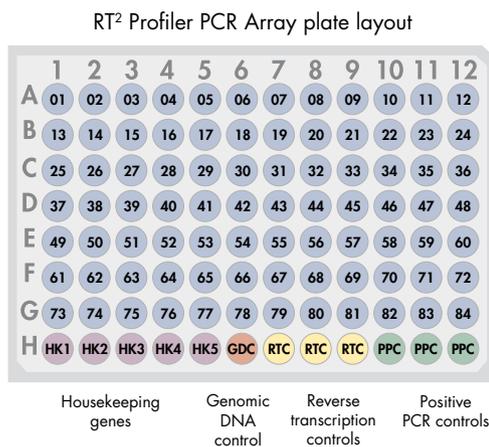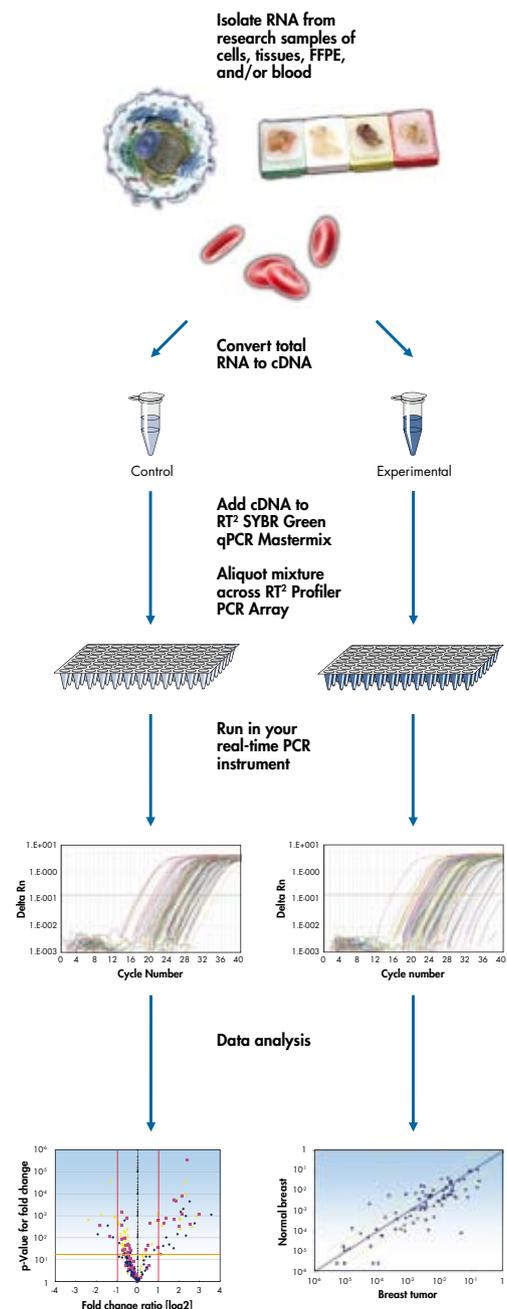
RT² Profiler PCR Array plate layout



Figure 1. Each well in an RT² Profiler PCR Array measures the expression of a gene related to a pathway or disease state. A typical 96-well format is shown. This is also available in a 384-well plate, 100-well disc, and 96x96 chip format.
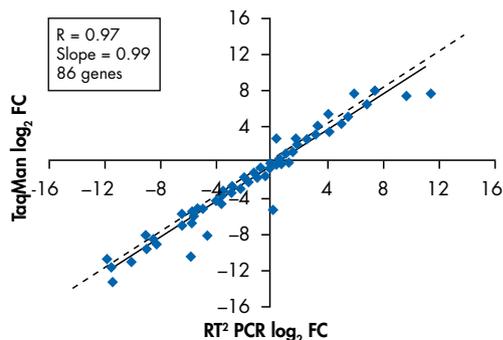
**Figure 2. Comparable biological results.*** Gene expression analysis was compared between RT² Profiler PCR Arrays (SYBR Green-based) and the TaqMan® platform. Regression analysis of fold differences, with data normalized against POLR2A, demonstrate that both platforms yield similar biological results.
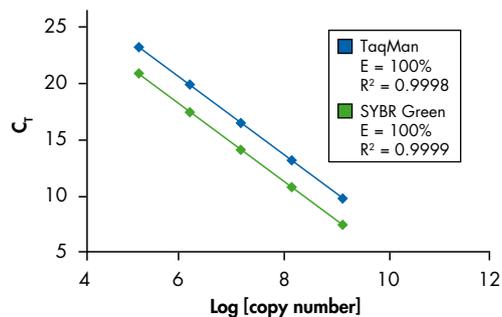


**Figure 3. Sensitivity with RT² SYBR Green versus TaqMan chemistry.*** PCR amplicons detected using the same primer pair with or without TaqMan probes in either SYBR Green or TaqMan chemistry. SYBR green chemistry yields earlier $C_t$s for each dilution, demonstrating better sensitivity than TaqMan chemistry.

**Figure 4. Stress and Toxicity PathwayFinder RT² Profiler PCR Array uncovered distinct gene expression profiles associated with liver toxicity caused by 3 PPARγ agonists.** RNA from HepG2 cells treated with three different glitazone PPARγ agonists for type 2 diabetes mellitus was characterized, and the results were compared to that of a vehicle (DMSO) control. The drug withdrawn due to idiosyncratic liver toxicity (Rezulin), induces very different changes in the expression of stress-related genes than two safer drugs still on the market (Avandia and Actos).
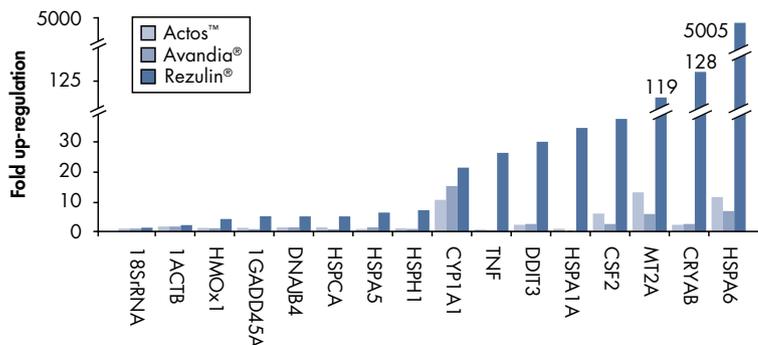


**Figure 5. Common Cytokine RT² Profiler PCR Array identified 23 up-regulated and 6 down-regulated genes following PBMC stimulation.** Triplicate total RNA samples from human peripheral blood mononuclear cells (either untreated or stimulated with 50 ng/ml PMA and 1 mg/ml ionomycin for 6 hours) were characterized with the human Common Cytokine RT² Profiler PCR Array. Twenty-three cytokine genes are up-regulated (> 5-fold, p < 0.0005) including interleukins, colony stimulating factors, and TNF ligands after 6 hours of stimulation. Six interleukin and TNF ligand genes are down-regulated under the same conditions.
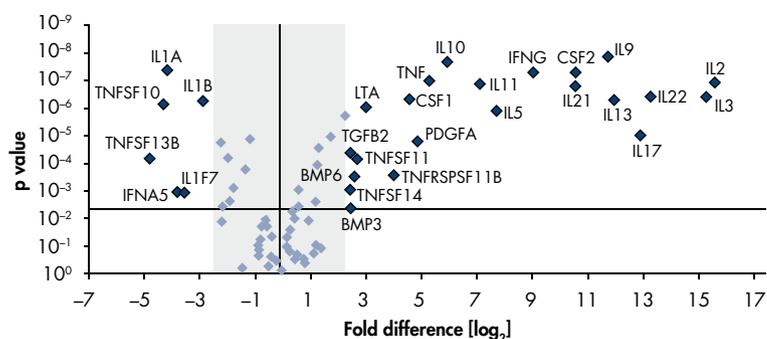


**Figure 6. Relative fold change between disorganized and organized colonies using the Angiogenesis RT² Profiler PCR Array.[†]** RNA isolated from unorganized T4-2 cells treated with a control antibody (IgG) or reverted to an organized colony by blocking EGFR signaling (mAb225) was reverse transcribed and relative gene expression data was obtained using the human Angiogenesis RT² Profiler PCR Array. The expression profile of 84 genes relevant to angiogenesis as well as 5 housekeeping genes were assayed. Fold change calculations were done using SABiosciences' data analysis software which automatically calculates the fold change in gene expression between the treated and control groups.
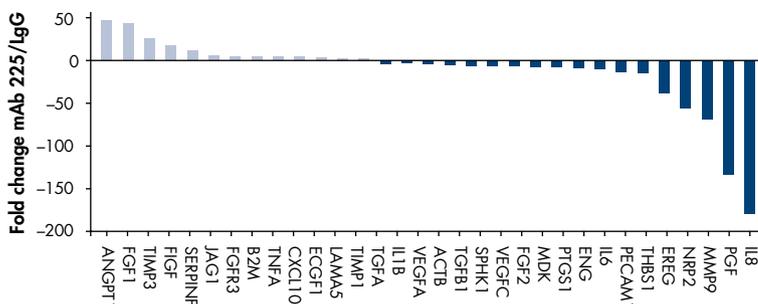
\* Arikawa, E., et al. (2008) Cross-platform comparison of SYBR Green real-time PCR with TaqMan PCR, microarrays and other gene expression measurement technologies evaluated in the MicroArray Quality Control (MAQC) study. BMC Genomics **9**, 378.

† Chen, A., et al. (2009) Endothelial cell migration and vascular endothelial growth factor expression are the result of loss of breast tissue polarity. Cancer Research **69**, 6721.

# How the RT² Profiler PCR Array system works

RT² Profiler PCR Arrays are a complete system for pathway-focused gene expression analysis. From sample preparation to data analysis, the RT² Profiler PCR Array system includes four components that guarantee high-quality, reproducible, and reliable gene expression data.

Integral to the performance of the RT² Profiler PCR Array system is a proprietary set of control elements that enhance the reliability of your data and serve as a guarantee for performance over time. These elements allow researchers to quickly assess the quality of their data by determining if samples were contaminated with genomic DNA (gDNA), the quality of the reverse transcription reaction, and real-time PCR efficiency. Each component of the RT² Profiler PCR Array system contributes to these quality control elements by incorporating an interlocked system for comprehensive monitoring of each step of the process.

■ **RT² Profiler PCR Arrays**

Each pathway-focused RT² Profiler PCR Array includes 89 wet bench verified RT² qPCR Primer Assays (including 5 housekeeping genes) and a proprietary control panel.

■ **RT² SYBR Green qPCR Mastermixes**

A unique formulation of buffers that co-evolved with the primer design algorithm provides high amplification efficiencies. Available with reference dyes (ROX, Fluorescein or without).

■ **RT² First Strand Kit**

An external RNA control detected by the RT² Profiler PCR Array tests the quality of input RNA. It also features a proprietary genomic DNA elimination buffer essential for eliminating residual gDNA, ensuring specific detection of mRNA.

■ **Free data analysis software**

The power of the RT² Profiler PCR Array to assess the expression of a pathway-focused set of genes over a wide range of detection yields an abundance of data. With our free RT² Profiler PCR Array data analysis tool, go from raw $C_T$ values to fold change results displayed in a variety of formats (scatter plots, volcano plots, clustergram) in a matter of minutes.
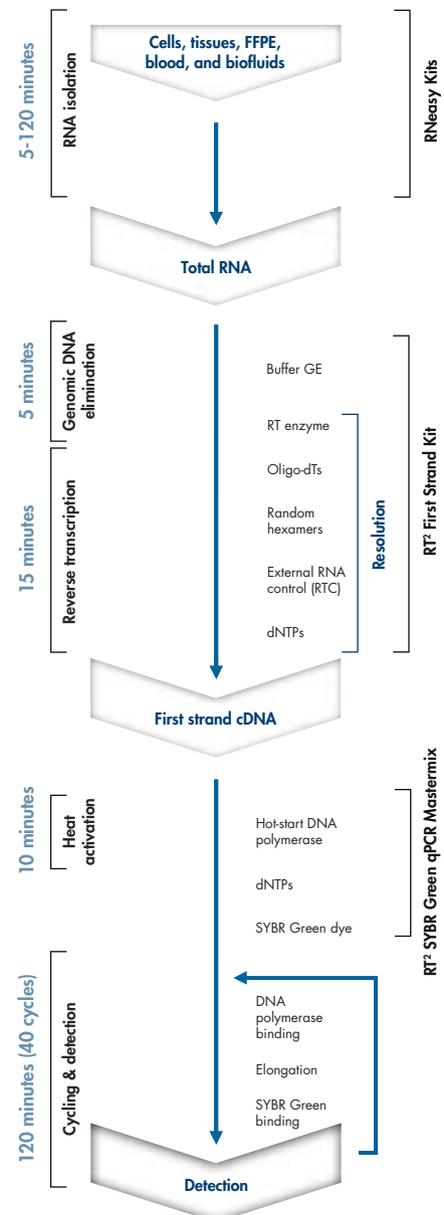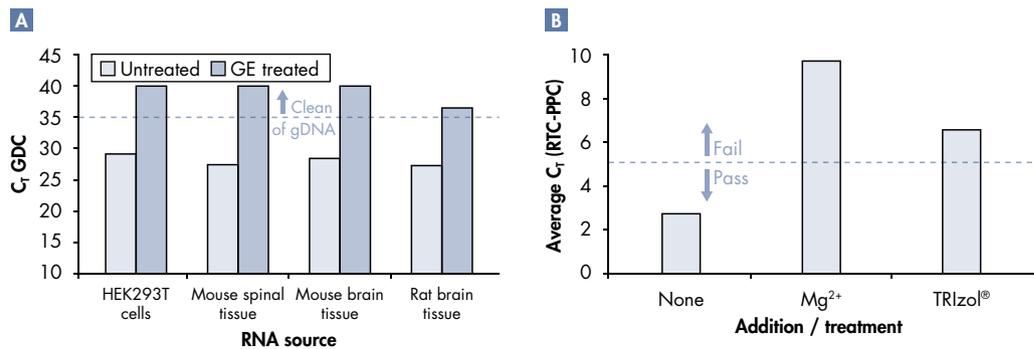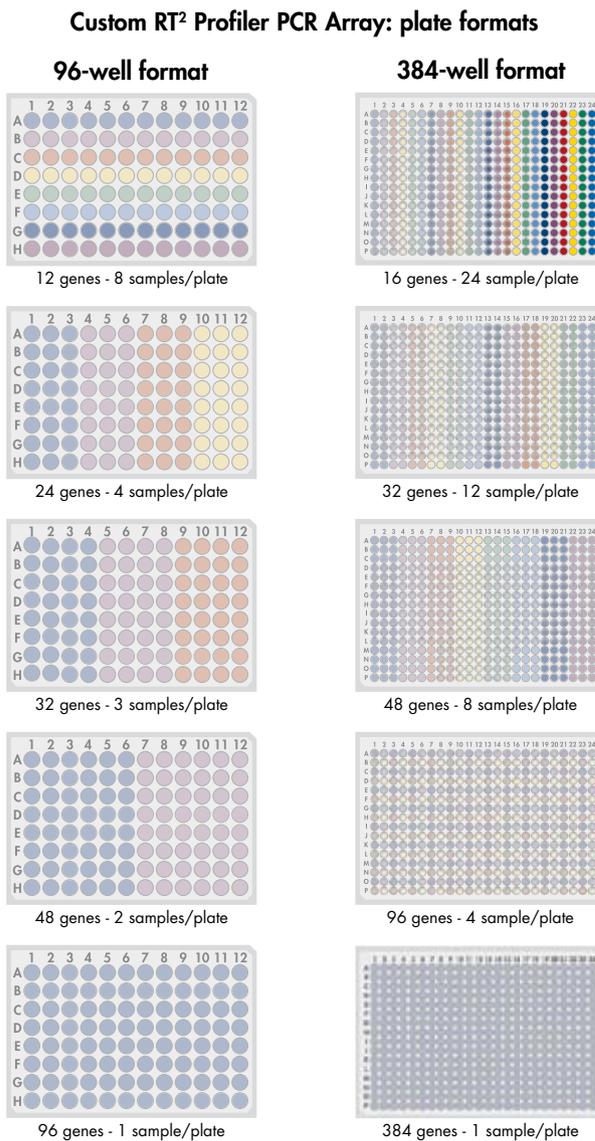
**Figure 7.** [A] **Elimination of genomic DNA contamination.** RNA from HEK 293T cells, mouse spinal tissue, mouse brain tissue, or rat brain tissue was characterized on RT² Profiler PCR Arrays before (light blue bars) and after (dark blue bars) treatment with gDNA Elimination Buffer from the RT² First Strand Kit. [B] **Monitoring inhibition in reverse transcription.** Human universal RNA was added with magnesium salt to simulate RNA degradation or added with TRIzol® reagent to simulate contamination that inhibits enzyme activity. RT² First Strand Kit was used for cDNA synthesis.

## Custom RT² Profiler PCR Array: plate formats

### 96-well format



12 genes - 8 samples/plate



24 genes - 4 samples/plate



32 genes - 3 samples/plate



48 genes - 2 samples/plate



96 genes - 1 sample/plate

### 384-well format



16 genes - 24 sample/plate



32 genes - 12 sample/plate



48 genes - 8 samples/plate



96 genes - 4 sample/plate



384 genes - 1 sample/plate

## What are custom RT² Profiler PCR Arrays?

Custom RT² Profiler PCR Arrays employ a high-throughput approach for profiling the expression of your genes of interest. Choose from any gene in the human, mouse, rat, rhesus macaque, drosophila, or dog genomes (up to 384 different genes). Whether your interests are in biomarker discovery, microarray followup, drug development, disease characterization, or signal transduction mechanisms, custom RT² Profiler PCR Arrays enable focused expression analysis on your genes of interest.

## Why custom RT² Profiler PCR Arrays?

- **Performance:** Each assay in a custom RT² Profiler PCR Array is designed and wet bench-verified using a set of rigorous parameters to insure the genes in your sample across a wide dynamic range are reproducibly recognized and quantified.

- **Flexibility:** Custom RT² Profiler PCR Arrays are available in a number of easy-to-use formats for quick sample loading and data analysis.

- **Turnaround time:** Submit your custom gene list and receive your custom RT² Profiler PCR Arrays in approximately 2 weeks.
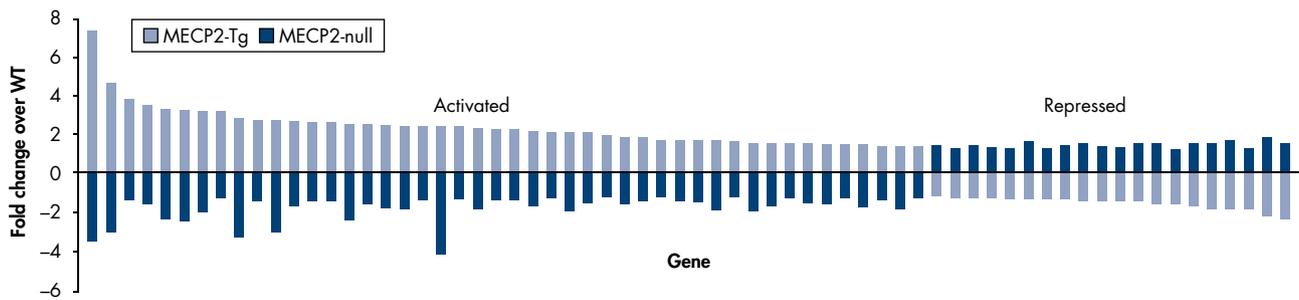
**Figure 8. Gene expression changes in hypothalamus of MECP2 mouse models.** Validation of expression changes for 66 genes by qPCR analysis. Gene expression levels from microarray analyses were validated in four MECP2-Tg males and four Mecp2-null males. Data is plotted as relative up-regulation (light blue) or down-regulation (dark blue) over wild-type ($P < 0.05$, t test). Each column represents a single gene, and represents data from four samples for each genotype.*

## 96-well plate[†] or 100-well disc custom RT² Profiler PCR Arrays

| Format | Number of arrays (minimum) |
|---|---|
| 8 genes, 12 samples/plate<br>12 genes, 8 samples/plate<br>16 genes, 6 samples/plate | 12 |
| 24 genes, 4 samples/plate<br>32 genes, 3 samples/plate<br>48 genes, 2 samples/plate<br>96 genes, 1 sample/plate | 24 |
| All formats | Per additional 12 arrays |

## 384-well custom RT² Profiler PCR Arrays

| Format | Number of arrays (minimum) |
|---|---|
| 8 genes, 48 samples/plate<br>12 genes, 32 samples/plate<br>16 genes, 24 samples/plate | 6 |
| 24 genes, 16 samples/plate<br>32 genes, 12 samples/plate<br>48 genes, 8 samples/plate<br>64 genes, 6 samples/plate<br>96 genes, 4 samples/plate<br>128 genes, 3 sample/plate | 6 |
| 192 genes, 2 samples/plate<br>384 genes, 1 sample/plate | 24 |
| All formats | Per additional 6 arrays |

## Custom RT² Profiler PCR Array: Rotor-Gene Q format

### 100-well disc format



12 genes - 8 sample/plate



96 genes - 1 sample/plate

| Modified RT² Profiler PCR Arrays | Number of arrays (minimum) |
|---|---|
| Add up to 4 genes to a cataloged RT² Profiler PCR Array (96-well, 100-well disc) | 24 |
| Add up to 4 genes to a cataloged RT² Profiler PCR Array (384-well) | 4 |

| RT² Profiler PCR Array accessories | Pack size | Catalog # |
|---|---|---|
| RT² PreAMP cDNA Synthesis Kit | 12 samples | 330451 |
| RT² PreAMP Pathway Primer Mixes (pathway focused) | 12 samples | 330241 |

* Chahrour, M., et al. (2008) MeCP2, A key contributor to neurological disease, activates and represses transcription. Science **320**, 1224.
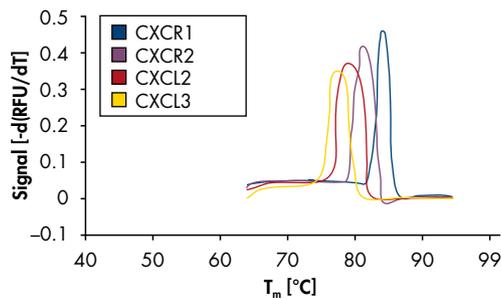
† Also available in 96x96 Fluidigm® BioMark format.

**Figure 9. RT² Profiler PCR Arrays amplify a single gene-specific product in every reaction.** Universal total RNA was characterized for four chemokine and chemokine receptors using RT² qPCR Primer Assays, followed by a dissociation (melt) curve analysis. RT² Profiler PCR Arrays specifically detect individual genes despite the expression of related gene family members in the same RNA sample.
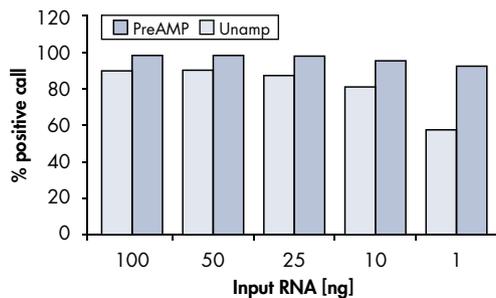


**Figure 10. RT² Profiler PCR Arrays detect as little as 1 ng of RNA.** Different amounts of universal total RNA were characterized using the Human Inflammatory Cytokines and Receptors RT² Profiler PCR Array (PAHS-011) with or without PreAMP. The percentage of detectable genes was calculated for each RNA amount, with 1 ng RNA analysis enabled with the new pathway-focused PreAMP technology.
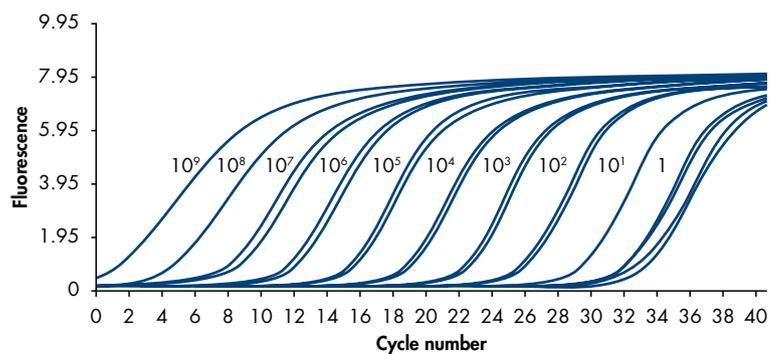
RT² Profiler PCR Arrays are used and trusted by thousands of research scientists for pathway-focused gene expression analysis. Several factors, including the RT² qPCR Primer Assay design algorithm, the proprietary control panel, and the strict manufacturing and quality control procedures, ensure the outstanding performance and reliability of our RT² Profiler PCR Arrays. Each RT² Profiler PCR Array and every RT² qPCR Primer Assay is wet bench-verified to guarantee their performance, with results demonstrating several performance parameters illustrated here.

## Distinct specificity

The complete RT² Profiler PCR Array system, with high quality input RNA, is guaranteed to yield single bands without primer dimers or other secondary products. The proprietary primer design algorithm incorporates more than 10 thermodynamic and sequence alignment criteria, and our wet bench verification provides confidence that every real-time qPCR assay accurately represents the expression of the queried gene. Over 20,000 gene-specific RT² qPCR Primer Assays have been designed and shipped to satisfied customers.

## High sensitivity and wide dynamic range

A key benefit of using pathway-focused RT² Profiler PCR Arrays for gene expression analysis is that genes that are over expressed can be measured as reliably as those that are under expressed. The complete RT² Profiler PCR Array system yields > 85% positive call with 25 ng – 5 µg RNA or > 90% with as little as 1 ng PreAMP RNA with the RT² PreAMP System. The 8-log wide dynamic range provided by real-time PCR is unparalleled when comparing a pathway-focused gene panel of varying expression levels across a variety of samples.

**Figure 11. RT² Profiler PCR Arrays detect RNA across a wide dynamic range.** Ten-fold serial dilutions of human CHRNA5 were characterized with the respective RT² qPCR Primer Assay.

## Gene expression analysis from FFPE samples

An innovative solution enabling the accurate qRT-PCR analysis of formalin-fixed paraffin-embedded (FFPE) samples. The RT² PreAMP technology utilizes multiplex tandem PCR to preamplify gene-specific cDNA with minimal bias. This kit is intended for preamplification of first-strand cDNA from fragmented total RNA from FFPE samples for gene expression analysis with RT² Profiler PCR Arrays.

The combination of a simplified RNA extraction and a high-fidelity amplification process maximizes recovery of RNA. RT² Profiler PCR Arrays facilitate easy and reliable expression analysis of genes associated with a biological pathway or a disease state from FFPE samples.


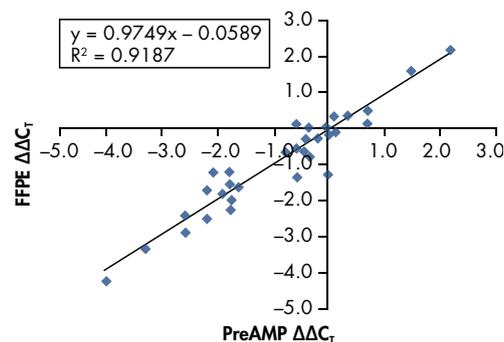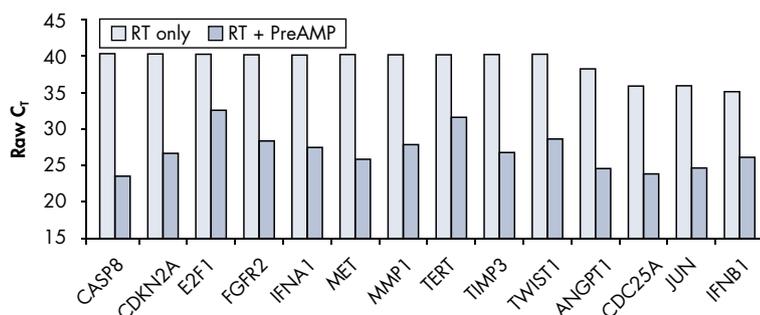
$$y = 0.9749x - 0.0589$$
$$R^2 = 0.9187$$

**Figure 12. Highly comparable gene expression fold change results between FFPE preamplified and unamplified samples.** RNA extracted from FFPE spleen and intestine samples were extracted and converted to cDNA with and without preamplification. All 4 cDNAs were analyzed on the Human Cancer PathwayFinder RT² Profiler PCR Array. The $\Delta\Delta C_T$ comparison and genes with raw $C_T$ values lower than 33 in both unpreamplified spleen and intestine samples are presented.

## Benefits of RT² PreAMP system for FFPE samples

- **Quick and efficient:** High quality and high-yield total RNA from FFPE samples with RNeasy FFPE Mini Kit

- **Superior sensitivity:** RT² PreAMP protocol significantly enhances qRT-PCR detection sensitivity for FFPE samples

- **Easy workflow:** Simple procedure and robust performance

## RT² PreAMP performance

- Increased positive call rate from FFPE samples

- Increased detection of genes previously classified as "absent"

- Unbiased amplification of preamplified genes

- Faithful conservation of biological changes

**Figure 13. Genes extracted from FFPE samples previously classified as "absent" are now detectable after RT² PreAMP preamplification.** RNA was extracted from FFPE spleen sample (human) kit and reverse transcribed to cDNA using RT² preamplification (dark blue bars) and without PreAMP (light blue bars). Results of the Human Cancer PathwayFinder RT² Profiler PCR Array showed 55% of unpreamplified genes were virtually undetectable with no genes in the 10-20 $C_T$ range. Preamplified genes with $C_T$ values > 30, shift into the reliably quantitative range ($C_T$ = 10-30).



## Ordering Information

| Product | Cat. no. |
|---|---|
| RT² PreAMP cDNA Synthesis Kit | 330451 |
| RT² PreAMP Pathway Primer Mixes for all RT² Profiler PCR Arrays | 330241 |
| RT² PreAMP Primer Mixes for custom RT² Profiler PCR Arrays | 330141 |
| RT² Profiler PCR Arrays | Varies |
| RT² SYBR Green qPCR Mastermixes | Varies |
| RNeasy FFPE Kit (50) | 73504 |

## Free web-based RT² Profiler PCR Array data analysis software

This integrated web-based software package for the RT² Profiler PCR Array system automatically performs all $\Delta\Delta C_T$ based fold-change calculations from your uploaded raw threshold cycle data. Simply providing the array's catalog number annotates the results to the correct gene list. The web portal delivers results not only in a tabular format but also in scatter, volcano, cluster-gram, and multi-group plots. Perform any pair-wise comparison between groups of experimental replicates by defining your own fold-change and statistical significance thresholds, or compare all of the groups side-by-side. The web portal also helps to correctly interpret the genomic DNA, reverse transcription efficiency, and positive PCR control well data. Make your pathway-focused gene expression analysis quick and painless with the RT² Profiler PCR Array system and the RT² Profiler PCR Array Data Analysis Suite.

- **Simple:** Just upload your data and define your parameters*
- **Convenient:** No downloading or installation required
- **Publication-ready output:** Export all results as free Excel® files or png image files

*Excel-based data analysis templates are available from our website.*

## Instructions

1. Upload your data in a simple Excel file format.

2. Define your housekeeping genes and experimental groups.

3. Choose an automatically generated data analysis result

- Take a test run with pre-loaded sample data set today:
  www.SABiosciences.com/pcrarraydataanalysis.php

- Join our next live webinar entitled: "PCR Array Data Analysis Tutorial" at:
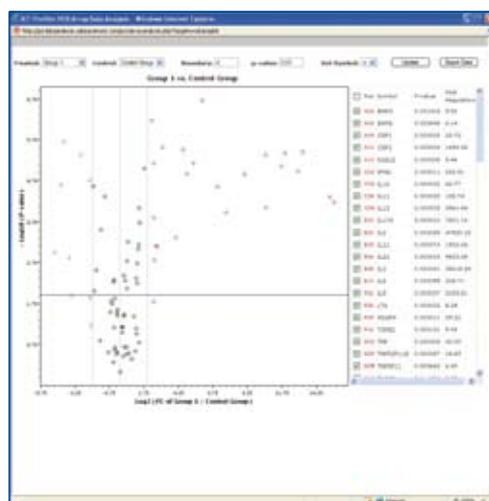  www.SABiosciences.com/seminarlist.php



**Figure 14. The volcano plot indicates the statistical significance of gene expression changes.** The x-axis plots the log2 of the fold-differences, while the y-axis plots their p-values based on student's t-test of your replicate raw $C_T$ data. The blue and red symbols outside the gray area conveniently have the same meaning as the scatter plot. Symbols in the volcano plot above the dashed line readily identify fold-differences at least as statistically significant as a threshold that can be defined.

## What are RNeasy Kits?

RNeasy Kits are a proven technology for rapid and convenient purification of high-quality RNA. Reproducible yields of intact RNA with high Agilent® RIN (RNA integrity number) values are obtained, enabling reliable results in downstream applications such as real-time RT-PCR. Kits are available for cells and easy-to-lyse tissues as well as for more challenging samples, such as fiber-rich or fatty tissues, fine needle aspirates, and cryosections.

## Why use RNeasy Kits?

When purifying RNA, it is important to use a method that maintains RNA integrity and removes contaminants. Degradation of RNA makes reliable analysis of gene expression impossible, while the presence of contaminants in the purified RNA can inhibit enzymes in downstream applications such as real-time RT-PCR and microarray analysis. RNeasy Kits overcome these challenges through the combination of a specialized lysis buffer and silica-membrane technology.

## How do RNeasy Kits work?

Biological samples are first lysed in a lysis buffer that contains a guanidine salt, which fully denatures RNases to prevent RNA degradation. RNA is then specifically bound to a silica membrane, either in an RNeasy spin column or the well of an RNeasy 96 plate. Other cellular material is efficiently washed away using a series of wash buffers before pure, intact RNA is eluted in RNase-free water.
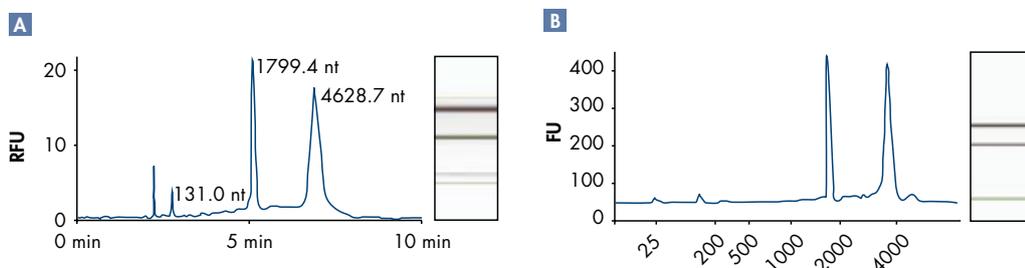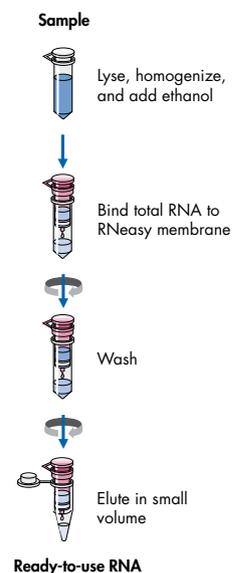
**Sample**

Lyse, homogenize, and add ethanol

Bind total RNA to RNeasy membrane

Wash

Elute in small volume

**Ready-to-use RNA**

Figure 15. **Highly intact RNA.** RNA was purified from Jurkat cells using the RNeasy Mini Kit. The purified RNA was analyzed on the [A] QIAxcel® system (ratio of 28S to 18S rRNA: 1.55) and [B] Agilent 2100 Bioanalyzer (ratio of 28S to 18S rRNA: 1.7). A high RNA integrity number (RIN) of 9.6 was obtained, indicating highly intact RNA.

## Ordering Information

| Product | Contents | Cat. no. |
| --- | --- | --- |
| RNeasy Mini Kit (50)* | For purification of RNA from cells & easy-to-lyse tissues | 74104 |
| RNase-Free DNase Set (50) | For DNase digestion RNA purification | 79254 |
| RNeasy Fibrous Tissue Mini Kit (50)* | For purification of RNA from fiber-rich tissues | 74704 |
| RNeasy Plus Universal Mini Kit (50)* | For purification of RNA from all tissue types | 73404 |
| RNeasy 96 Kit (12) | For purification of RNA from cells in 96-well format | 74182 |
| QIAzol® Lysis Reagent (200 ml) | For lysis of fatty and standard tissues before RNA isolation | 79306 |

* Automatable on the QIAcube. Find out more at www.qiagen.com/goto/QIAcube.

## What is sample disruption?

Effective disruption and homogenization of a biological sample is an absolute requirement for all RNA purification procedures. Disruption releases the RNA contained in a sample, while homogenization reduces sample viscosity to facilitate subsequent RNA purification.
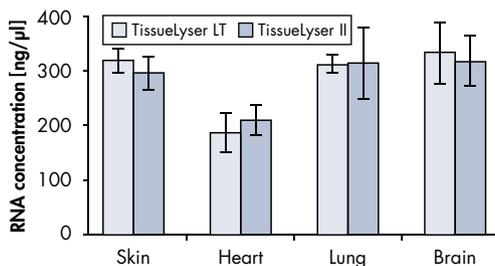


**Figure 16. Effective tissue disruption.** Various rat tissues were disrupted using the TissueLyser LT or TissueLyser II. RNA was purified from 20 mg samples on the QIAcube® using the RNeasy Fibrous Tissue Mini Kit (skin, heart, and lung) or RNeasy Lipid Tissue Mini Kit (brain). RNA was eluted in a volume of 50 µl, and concentration was determined using a spectrophotometer.

## Why use QIAGEN sample disruption products?

QIAGEN provides a range of technologies for disruption and homogenization — from QIAshredder spin columns for fast and simple homogenization of cell lysates to TissueRuptor and TissueLyser systems for mechanical disruption and homogenization of tougher tissue samples at a range of throughputs. TissueRuptor and TissueLyser systems deliver fast and effective disruption, and replace tedious and time-consuming methods such as disruption using a mortar and pestle.

## How do QIAGEN sample disruption products work?

The QIAshredder is a biopolymer-shredding system in a spin-column format. Cell lysate is applied to a QIAshredder spin column, which is then briefly centrifuged to homogenize the lysate.

The TissueRuptor is a handheld device that provides simultaneous disruption and homogenization using TissueRuptor Disposable Probes, which contain a blade that rotates at very high speeds. As the probes are both disposable and transparent, the risk of cross-contamination is minimized and the sample disruption process can be visually monitored. Use of disposable probes also saves time as there is no need to clean the same probe after disrupting each sample.

TissueLyser instruments are bead mills that simultaneously disrupt and homogenize samples through high-speed shaking with grinding beads in plastic tubes. Using an adapter that holds several tubes, the instruments disrupt multiple samples at the same time — up to 12 samples with the TissueLyser LT, and up to 48 or 192 samples with the TissueLyser II.

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAshredder (50) | For homogenization of cell lysates | 79654 |
| TissueRuptor® | For disruption of individual samples | 9001271 |
| TissueRuptor Disposable Probes (25) | Disposable probes for use with the TissueRuptor | 990890 |
| TissueLyser LT | For disruption of up to 12 samples | 85600 |
| TissueLyser LT Adapter, 12-tube | For purification of RNA from cells in 96-well format | 69980 |
| TissueLyser II | For disruption of up to 48 or 192 samples | 85300 |
| TissueLyser Adapter Set 2 x 24 | Adaptor set for use with the TissueLyser II; holds 48 tubes | 69982 |

## What is RNA stabilization?

Once a biological sample is harvested, its RNA becomes extremely unstable. The RNA is degraded by RNases, and gene induction or downregulation triggered by sample manipulation will also occur. Immediate stabilization of cellular RNA to preserve mRNA levels is critical for accurate gene expression analysis.

RNA stabilization is usually achieved by rapidly freezing samples in liquid nitrogen or on dry ice. However, the use of such chemicals is hazardous, and care should be taken to avoid thawing of samples prior to sample disruption and RNA purification.

## Why use QIAGEN RNA stabilization products?

QIAGEN provides a broad range of reagents for convenient stabilization of RNA in cells, tissues, blood, and saliva at room temperature. The use of hazardous liquid nitrogen or dry ice to freeze samples is avoided. Samples are simply submerged in the reagents to immediately preserve the gene expression profile, and can then be conveniently handled and transported at ambient temperature prior to RNA purification. For further convenience, stabilization reagents are also available as part of QIAGEN kits for RNA purification.
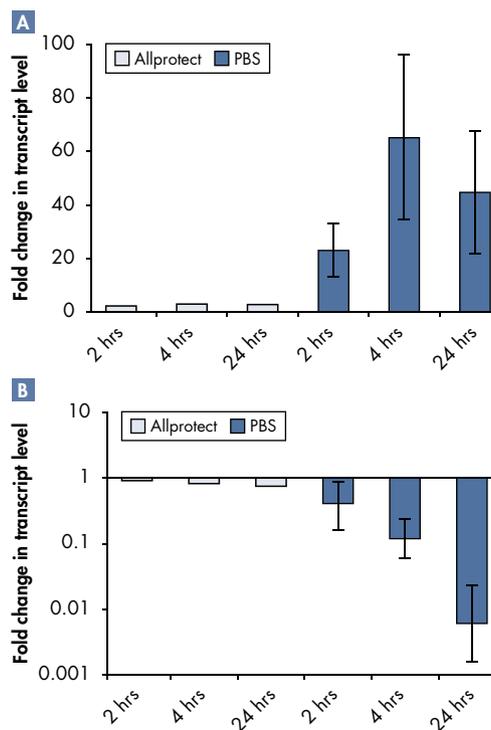
**Figure 17. Effective RNA stabilization.** Rat tissues were stored at 25°C for 2–24 hours in Allprotect Reagent or PBS prior to real-time RT-PCR analysis. **A** Rat lung tissue was analyzed for c-fos expression. **B** Rat intestine tissue was analyzed for Madh7 expression. Transcript levels relative to those in liquid nitrogen stabilized tissues were calculated. Changes in transcript levels were prevented by Allprotect Reagent.

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Allprotect Tissue Reagent (100 ml) | For stabilization of RNA, DNA, & protein in tissues | 76405 |
| AllPrep® DNA/RNA Mini Kit (50) | For simultaneous purification of DNA and RNA | 80204 |
| RNA*later*® RNA Stabilization Reagent (250 ml) | For stabilization of RNA in tissues | 76106 |
| RNeasy Protect Mini Kit (50) | For stabilization of RNA in tissues and RNA purification | 74124 |
| RNAprotect® Cell Reagent (250 ml) | For stabilization of RNA in cells | 76526 |
| RNAprotect Animal Blood Tubes (50 x 100 µl) | For collection of 100 µl animal blood with RNA stabilization | 76544 |
| RNeasy Protect Animal Blood Kit (50) | For purification of RNA from blood collected in RNAprotect Animal Blood Tubes | 73224 |
| miRNeasy Protect Animal Blood Kit (50) | For purification of RNA, including miRNA, from blood collected in RNAprotect Animal Blood Tubes | 217304 |

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.

| Research area | Apoptosis Research | Biomarker Research | Cancer Research | Cell Cycle Research |
|---|---|---|---|---|
| **RT² Profiler PCR Array listing**<br><br>For catalog numbers, see page 2 or visit:<br>www.SABiosciences.com/ArrayList.php | Apoptosis | Alzheimer's Disease | Angiogenesis | Apoptosis |
| | Autophagy | Angiogenesis | Apoptosis | Autophagy |
| | Cancer PathwayFinder | Breast Cancer and Estrogen Receptor Signaling | Breast Cancer and Estrogen Receptor Signaling | Cancer PathwayFinder |
| | Cell Cycle | Cancer PathwayFinder | Cancer Drug Resistance and Metabolism | Cell Cycle |
| | DNA Damage Signaling Pathway | Cell Surface Markers | Cancer PathwayFinder | DNA Damage Signaling Pathway |
| | DNA Repair | Dendritic and Antigen Presenting Cell | Cell Cycle | DNA Repair |
| | Endothelial Cell Biology | Epigenetic Chromatin Modification Enzymes | DNA Damage Signaling Pathway | Epithelial to senchymal Transition (EMT) |
| | Heat Shock Proteins | Epigenetic Chromatin Remodeling Factors | EGF/PDGF Signaling Pathway | MAP Kinase Signaling Pathway |
| | NFκB Signaling Pathway | Epithelial to esenchymal Transition (EMT) | Epithelial to Mesenchymal Transition  (EMT) | mTOR Signaling |
| | Oxidative Stress and Antioxidant Defense | Extracellular Matrix and Adhesion Molecules | MAP Kinase Signaling Pathway | Neurogenesis and Neural Stem Cell |
| **Try RT² Profiler PCR Arrays** | p53 Signaling Pathway | Glucose Metabolism | p53 Signaling Pathway | NFκB Signaling Pathway |
| | PI3K-AKT Signaling Pathway | Hematopoietic Stem Cells and Hematopoiesis | PI3K-AKT Signaling Pathway | p53 Signaling Pathway |
| | Stress and Toxicity PathwayFinder | Homeobox (HOX) Genes | Protein Phosphatases | PI3K-AKT Signaling Pathway |
| | TNF Ligand and Receptor | Mesenchymal Stem Cell | TGFβ BMP Signaling Pathway | Protein Phosphatases |
| | Tumor Suppressor Genes | Stem Cell | Tumor Metastasis | Signal Transduction PathwayFinder |
| **Risk-free starter pack offer details:**<br>www.SABiosciences.com/PCRguide.php | Ubiquitination Pathway | T-cell and B-cell Activation | Tumor Suppressor Genes | Transcription Factors |
| | Unfolded Protein Response | Th1-Th2-Th3 | WNT Signaling Pathway | Ubiquitination Pathway |

RT² Profiler PCR Array free data analysis tool, see page 10. http://sabiosciences.com/pcrarraydataanalysis.php

| Inflammation Research | ECM/Adhesion Research | Neuroscience Research | Signal Transduction Research | Stem Cell Research | Toxicology/Drug ADME Research |
|---|---|---|---|---|---|
| Chemokines & Receptors | Angiogenic Growth Factors & Angiogenesis Inhibitors | Alzheimer's Disease | cAMP/Ca2+ Signaling PathwayFinder | Adipogenesis | Cancer Drug Resistance and Metabolism |
| Common Cytokine | Atherosclerosis | Apoptosis | EGF/PDGF Signaling Pathway | Dendritic and Antigen Presenting Cell | Cancer PathwayFinder |
| Inflammasomes | Chemokines and Receptors | Autophagy | G Protein Coupled Receptors | Embryonic Stem Cells | Cardiotoxicity |
| Inflammatory Cytokines and Receptors | Common Cytokine | Drug Transporters | GPCR Signaling PathwayFinder | Hedgehog Signaling Pathway | Cell Cycle |
| Inflammatory Response and Autoimmunity | Embryonic Stem Cells | Embryonic Stem Cells | Heat Shock Proteins | Hematopoietic Stem Cells and Hematopoiesis | DNA Damage Signaling Pathway |
| Interferon α, β Response | Endothelial Cell Biology | GPCR Signaling PathwayFinder | Hedgehog Signaling Pathway | Homeobox (HOX) Genes | Drug Metabolism |
| Interferon and Receptor | Extracellular Matrix and Adhesion Molecules | Heat Shock Proteins | Insulin Signaling Pathway | Lipoprotein Signaling and Cholesterol Metabolism | Drug Metabolism: Phase I Enzymes |
| JAK/STAT Signaling Pathway | Glycosylation | Hedgehog Signaling Pathway | JAK/STAT Signaling Pathway | Mesenchymal Stem Cell | Drug Metabolism: Phase II Enzymes |
| NFκB Signaling Pathway | MAP Kinase Signaling Pathway | Huntington's Disease | MAP Kinase Signaling Pathway | Neurogenesis and Neural Stem Cell | Drug Transporters |
| T Cell Anergy & Immune Tolerance | Mesenchymal Stem Cell | Hypoxia Signaling Pathway | mTOR Signaling | Neurotrophin & Receptors | GPCR Signaling PathwayFinder |
| T-cell and B-cell Activation | NFκB Signaling Pathway | Mesenchymal Stem Cell | NFκB Signaling Pathway | Notch Signaling Pathway | Hepatotoxicology |
| TGFβ BMP Signaling Pathway | Osteogenesis | Neurogenesis and Neural Stem Cell | Nuclear Receptors and Coregulators | Osteogenesis | Lipoprotein Signaling & Cholesterol Metabolism |
| Th17 for Autoimmunity and Inflammation | TGFβ BMP Signaling Pathway | Neuroscience Ion Channels and Transporters | PI3K-AKT Signaling Pathway | Stem Cell Signaling | Mitochondria |
| Th1-Th2-Th3 | TNF Ligand and Receptor | Neurotransmitter Receptors and Regulators | Signal Transduction PathwayFinder | T-cell and B-cell Activation | Molecular Toxicology 384HT |
| TNF Ligand and Receptor | Tumor Metastasis | Neurotrophin and Receptors | TGFβ BMP Signaling Pathway | Terminal Differentiation Marker | Nephrotoxicity |
| Toll-Like Receptor Signaling Pathway | VEGF Signaling | Nitric Oxide Signaling Pathway | Transcription Factors | TGFβ BMP Signaling Pathway | Oxidative Stress and Antioxidant Defense |
| Tumor Necrosis Factor (TNF) Ligand and Receptor | Wound Healing | Notch Signaling Pathway | Wnt Signaling Pathway | Wnt Signaling Pathway | Stress and Toxicity PathwayFinder |

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