# RT<sup>2</sup> Profiler PCR Arrays: Pathway Analysis



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

RT <sup>2</sup> Profiler PCR Array	accessory	Cat. no.
RT <sup>2</sup> First Strand Kit	12 samples	330401

Master Mix for RT <sup>2</sup> Profile	er PCR Arrays	Cat. no.
RT <sup>2</sup> SYBR® Green	2 arrays (96-well)	330520
ROX <sup>TM</sup> qPCR Mastermix	12 arrays (96-well)	330522
	24 arrays (96-well)	330523
	4 arrays (384-well)	330521
RT <sup>2</sup> SYBR Green	2 arrays (96-well)	330510
Fluor qPCR Mastermix	12 arrays (96-well)	330512
	24 arrays (96-well)	330513
	<u>4 arrays (384-well)</u>	330511
RT <sup>2</sup> SYBR Green qPCR	2 arrays (96-well)	330500
Mastermix	12 arrays (96-well)	330502
	24 arrays (96-well)	330503
	4 arrays (384-well)	330501

Popular RT <sup>2</sup> Profiler PCR Arrays for research	Cat. no.* SAP # / PCR Array #
Angiogenesis	330231 / <u>PAXX-024Y</u>
<u>Apoptosis</u>	330231 / <u>PAXX-012Y</u>
Autophagy	330231 / <u>PAXX-084Y</u>
Breast Cancer and Estrogen Receptor Signaling	330231 / <u>PAXX-005Y</u>
Cancer PathwayFinder	330231 / <u>PAXX-033Y</u>
Cell Cycle	330231 / <u>PAXX-020Y</u>
Chemokines and Receptors	330231 / <u>PAXX-022Y</u>
<u>Diabetes</u>	330231 / <u>PAXX-023Y</u>
<u>DNA Damage Signaling</u> <u>Pathway</u>	330231 / <u>PAXX-029Y</u>
<u>Drug Metabolism</u>	330231 / <u>PAXX-002Y</u>
EGF/PDGF Signaling Pathway	330231 / <u>PAXX-040Y</u>
Embryonic Stem Cells	330231 / <u>PAXX-081Y</u>
Endothelial Cell Biology	330231 / <u>PAXX-015Y</u>
Epigenetic Chromatin Modification Enzymes	330231 / <u>PAXX-085Y</u>
Epithelial to Mesenchymal Transition	330231 / <u>PAXX-090Y</u>
Extracellular Matrix and Adhesion Molecules	330231 / <u>PAXX-013Y</u>
GPCR Signaling Pathway	330231 / <u>PAXX-071Y</u>
Growth Factors	330231 / <u>PAXX-041Y</u>
Heat Shock Proteins	330231 / <u>PAXX-076Y</u>
<u>Hedgehog Signaling</u> <u>Pathway</u>	330231 / <u>PAXX-078Y</u>
Hematopoietic Stem Cells & Hematopoesis	330231 / <u>PAXX-054Y</u>
<u>Hepatoxicity</u>	330231 / <u>PAXX-093Y</u>
Hypoxia Signaling Pathway	330231 / <u>PAXX-032Y</u>
Inflammatory Cytokines and Receptors	330231 / <u>PAXX-011Y</u>
Innate and Adaptive Immune Response	330231 / <u>PAXX-052Y</u>
Interferon α,β Response	330231 / <u>PAXX-016Y</u>
JAK/STAT Signaling Pathway	330231 / <u>PAXX-039Y</u>
MAP Kinase Signaling Pathway	330231 / <u>PAXX-061Y</u>
Mitochondrial Energy Metabolism	330231 / <u>PAXX-008Y</u>
Nephrotoxicity	330231 / <u>PAXX-094Y</u>
NFκB Signaling Pathway	330231 / <u>PAXX-025Y</u>
Oxidative Stress and Antioxidant Defense	330231 / <u>PAXX-065Y</u>
p53 Signaling Pathway	330231 / <u>PAXX-027Y</u>
Wnt Signaling Pathway	330231 / <u>PAXX-043Y</u>

<sup>\*</sup> XX = species; Y = plate format.

## What are RT<sup>2</sup> Profiler PCR Arrays?

RT<sup>2</sup> 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 RT<sup>2</sup> 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 RT<sup>2</sup> Profiler PCR Arrays?

- Simplicity: The simplicity of RT<sup>2</sup> Profiler PCR Arrays makes routine expression profiling practical in any research laboratory with a realtime instrument.
- Performance: RT<sup>2</sup> 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: RT<sup>2</sup> Profiler PCR Arrays focus on profiling the genes relevant to the pathways or disease states important to your research.

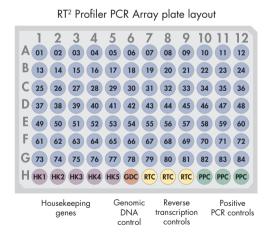
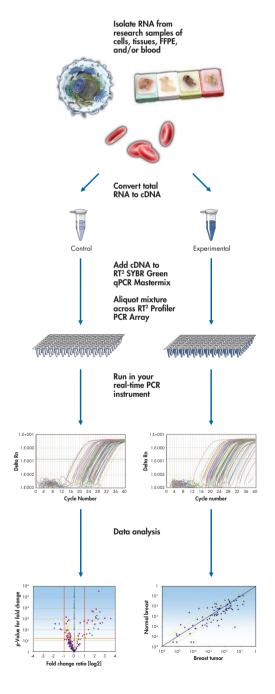
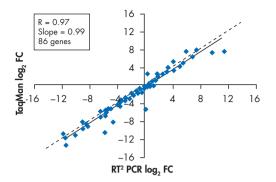


Figure 1. Each well in an RT<sup>2</sup> Profiler PCR Array measures the expression of a gene related to a pathway or disease state. A typical 96-well format is shown. This is also available in a 384-well plate, 100-well disc, and 96x96 chip format.





25 TaqMan E = 100% $R^2 = 0.9998$ 20 SYBR Green Ç E = 100% $R^2 = 0.9999$ 15 10 5 4 6 8 10 12 Log [copy number]

Figure 2. Comparable biological results.\* Gene expression analysis was compared between RT<sup>2</sup> 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 RT<sup>2</sup> 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<sub>T</sub>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 PPARy agonists. RNA from HepG2 cells treated with three different glitazone PPARy 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).

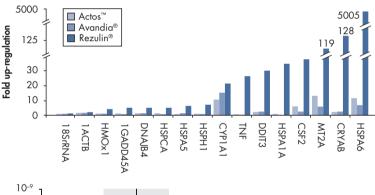


Figure 5. Common Cytokine RT² Profiler PCR Array identified 23 up-regulated and 6 down-regulated genes following PBMC stimulation. Triplicate total RNA samples from human peripheral blood mononuclear cells (either untreated or stimulated with 50 ng/ml PMA and 1 mg/ml ionomycin for 6 hours) were characterized 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 ligand genes are down-regulated under the same conditions.

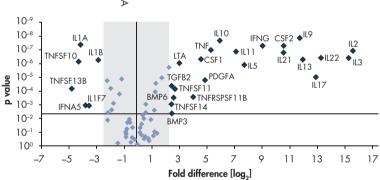
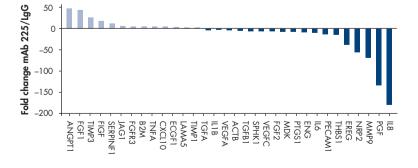


Figure 6. Relative fold change between disorganized and organized colonies using the Angiogenesis RT² Profiler PCR Array.† RNA isolated from unorganized T4-2 cells treated with a control antibody (IgG) or reverted to an organized colony by blocking EGFR signaling (mAb225) was reverse transcribed and 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.



<sup>\*</sup> 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.

<sup>†</sup> 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 RT<sup>2</sup> Profiler PCR Array system works

RT<sup>2</sup> Profiler PCR Arrays are a complete system for pathway-focused gene expression analysis. From sample preparation to data analysis, the RT<sup>2</sup> Profiler PCR Array system includes four components that guarantee high-quality, reproducible, and reliable gene expression data.

Integral to the performance of the RT<sup>2</sup> 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 RT<sup>2</sup> Profiler PCR Array system contributes to these quality control elements by incorporating an interlocked system for comprehensive monitoring of each step of the process.

## RT<sup>2</sup> Profiler PCR Arrays

Each pathway-focused RT<sup>2</sup> Profiler PCR Array includes 89 wet bench verified RT<sup>2</sup> qPCR Primer Assays (including 5 housekeeping genes) and a proprietary control panel.

#### RT<sup>2</sup> SYBR Green qPCR Mastermixes

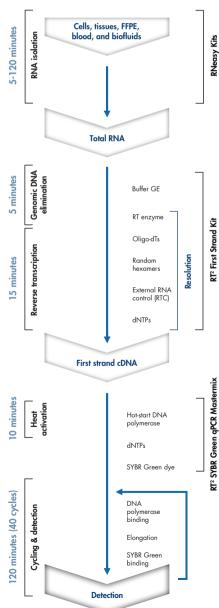
A unique formulation of buffers that co-evolved with the primer design algorithm provides high amplification efficiencies. Available with reference dyes (ROX, Fluorescein or without).

#### RT<sup>2</sup> First Strand Kit

An external RNA control detected by the RT<sup>2</sup> 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 RT $^2$  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 RT $^2$  Profiler PCR Array data analysis tool, go from raw  $C_{\scriptscriptstyle T}$  values to fold change results displayed in a variety of formats (scatter plots, volcano plots, clustergram) in a matter of minutes.



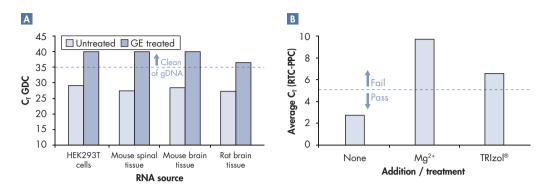
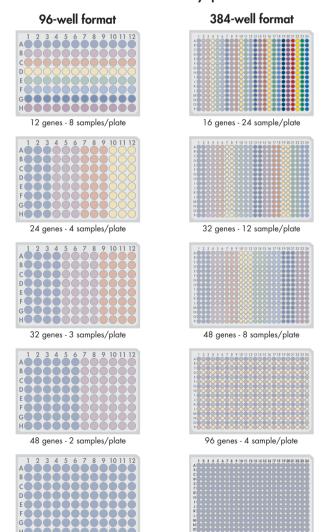


Figure 7. Elimination of genomic DNA contamination. RNA from HEK 293T cells, mouse spinal tissue, mouse brain tissue, or rat brain tissue was characterized on RT2 Profiler PCR Arrays before (light blue bars) and after (dark blue bars) treatment with gDNA Elimination Buffer from the RT2 First Strand Kit. 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 RT<sup>2</sup> Profiler PCR Array: plate formats



# What are custom RT<sup>2</sup> Profiler PCR Arrays?

Custom RT<sup>2</sup> 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 RT<sup>2</sup> Profiler PCR Arrays enable focused expression analysis on your genes of interest.

# Why custom RT<sup>2</sup> Profiler PCR Arrays?

- Performance: Each assay in a custom RT<sup>2</sup> 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 RT<sup>2</sup> 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 RT<sup>2</sup> Profiler PCR Arrays in approximately 2 weeks.

96 genes - 1 sample/plate

384 genes - 1 sample/plate

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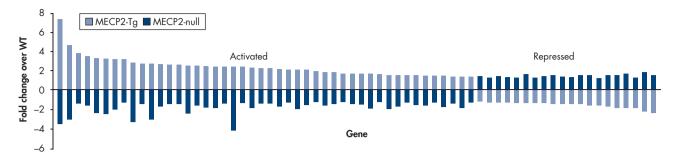


Figure 8. Gene expression changes in hypothalamus of MECP2 mouse models. Validation of expression changes for 66 genes by qPCR analysis. Gene expression levels from microarray analyses were validated in four MECP2-Tg males and four Mecp2-null males. Data is plotted as relative up-regulation (light blue) 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<sup>†</sup> or 100-well disc custom RT<sup>2</sup> Profiler PCR Arrays

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

### 384-well custom RT<sup>2</sup> Profiler PCR Arrays

Format	Number of arrays (minimum)
8 genes, 48 samples/plate	
12 genes, 32 samples/plate	6
16 genes, 24 samples/plate	
24 genes, 16 samples/plate	
32 genes, 12 samples/plate	
48 genes, 8 samples/plate	6
64 genes, 6 samples/plate	0
96 genes, 4 samples/plate	
128 genes, 3 sample/plate	
192 genes, 2 samples/plate	24
384 genes, 1 sample/plate	
All formats	Per additional 6 arrays

Modified RT <sup>2</sup> Profiler PCR Arrays	Number of arrays (minimum)
Add up to 4 genes to a cataloged RT <sup>2</sup> Profiler PCR Array (96-well, 100-well disc)	24
Add up to 4 genes to a cataloged RT <sup>2</sup> Profiler PCR Array (384-well)	4

## Custom RT<sup>2</sup> Profiler PCR Array: Rotor-Gene Q format

#### 100-well disc format



12 genes - 8 sample/plate



96 genes - 1 sample/plate

RT <sup>2</sup> Profiler PCR Array accessories	Pack size	Catalog #
RT <sup>2</sup> PreAMP cDNA Synthesis Kit	12 samples	330451
RT <sup>2</sup> PreAMP Pathway Primer Mixes (pathway focused)	12 samples	330241

<sup>\*</sup> Chahrour, M., et al. (2008) MeCP2, A key contributor to neurological disease, activates and represses transcription. Science 320, 1224.

<sup>†</sup> Also available in 96x96 Fluidigm® BioMark format.

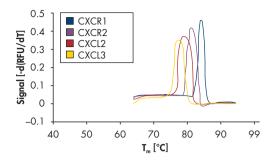


Figure 9. RT² Profiler PCR Arrays amplify a single genespecific product in every reaction. Universal total RNA was characterized for four chemokine and chemokine receptors using RT² qPCR Primer Assays, followed by a dissociation (melt) curve analysis. RT² Profiler PCR Arrays specifically detect individual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

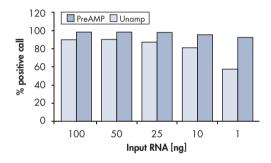


Figure 10. RT² Profiler PCR Arrays detect as little as 1 ng of RNA. Different amounts of universal total RNA were characterized using the Human Inflammatory Cytokines and Receptors RT² Profiler PCR Array (PAHS-011) with or without PreAMP. The percentage of detectable genes was calculated for each RNA amount, with 1 ng RNA analysis enabled with the new pathway-focused PreAMP technology.

Figure 11. RT<sup>2</sup> Profiler PCR Arrays detect RNA across a wide dynamic range. Ten-fold serial dilutions of human CHRNA5 were characterized with the respective RT<sup>2</sup> qPCR Primer Assay.

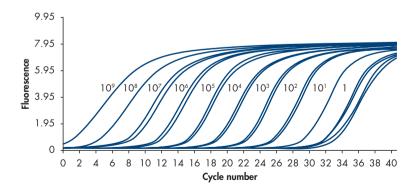
RT<sup>2</sup> Profiler PCR Arrays are used and trusted by thousands of research scientists for pathway-focused gene expression analysis. Several factors, including the RT<sup>2</sup> qPCR Primer Assay design algorithm, the proprietary control panel, and the strict manufacturing and quality control procedures, ensure the outstanding performance and reliability of our RT<sup>2</sup> Profiler PCR Arrays. Each RT<sup>2</sup> Profiler PCR Array and every RT<sup>2</sup> qPCR Primer Assay is wet bench-verified to guarantee their performance, with results demonstrating several performance parameters illustrated here.

## **Distinct specificity**

The complete RT<sup>2</sup> 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 RT<sup>2</sup> 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.



## Gene expression analysis from FFPE samples

An innovative solution enabling the accurate qRT-PCR analysis of formalin-fixed paraffin-embedded (FFPE) samples. The RT<sup>2</sup> 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 RT<sup>2</sup> Profiler PCR Arrays.

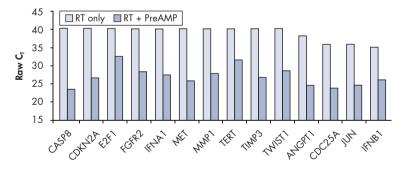
The combination of a simplified RNA extraction and a high-fidelity amplification process maximizes recovery of RNA. RT<sup>2</sup> Profiler PCR Arrays facilitate easy and reliable expression analysis of genes associated with a biological pathway or a disease state from FFPE samples.

## Benefits of RT<sup>2</sup> 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: RT<sup>2</sup> PreAMP protocol significantly enhances gRT-PCR detection sensitivity for FFPE samples
- **Easy workflow:** Simple procedure and robust performance

## RT<sup>2</sup> 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



 $\begin{array}{c} y = 0.9749 x - 0.0589 \\ R^2 = 0.9187 \\ \hline \\ 2.0 \\ \hline \\ 1.0 \\ \hline \\ -5.0 \\ \hline \\ -4.0 \\ \hline \\ -5.0 \\ \hline \\ \end{array}$ 

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  $\Delta\Delta C_{\rm T}$  comparison and genes with raw  $C_{\rm T}$  values lower than 33 in both unpreamplified spleen and intestine samples are presented.

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  $\rm C_T$  range. Preamplified genes with  $\rm C_T$  values > 30, shift into the reliably quantitative range ( $\rm C_T$  = 10-30).

## Ordering Information

Product	Cat. no.
RT <sup>2</sup> PreAMP cDNA Synthesis Kit	330451
RT <sup>2</sup> PreAMP Pathway Primer Mixes for all RT <sup>2</sup> Profiler PCR Arrays	330241
RT <sup>2</sup> PreAMP Primer Mixes for custom RT <sup>2</sup> Profiler PCR Arrays	330141
RT <sup>2</sup> Profiler PCR Arrays	<u>Varies</u>
RT <sup>2</sup> SYBR Green qPCR Mastermixes	<u>Varies</u>
RNeasy FFPE Kit (50)	<u>73504</u>

## Free web-based RT<sup>2</sup> Profiler PCR Array data analysis software

This integrated web-based software package for the RT $^2$  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 RT $^2$  Profiler PCR Array system and the RT $^2$  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

#### 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

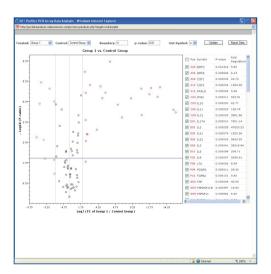


Figure 14. The volcano plot indicates the statistical significance of gene expression changes. The x-axis plots the log2 of the fold-differences, while the y-axis plots their p-values based on student's t-test of your replicate raw  $C_{\scriptscriptstyle T}$  data. The blue and red symbols outside the 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.

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<sup>\*</sup>Excel-based data analysis templates are available from our website.

## What are RNeasy Kits?

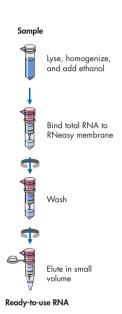
RNeasy Kits are a proven technology for rapid and convenient purification of high-quality RNA. Reproducible yields of intact RNA with high Agilent® RIN (RNA integrity number) values are obtained, enabling reliable results in downstream applications such as real-time RT-PCR. Kits are available for cells 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.



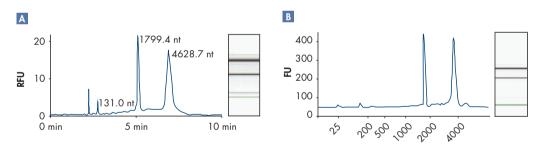


Figure 15. Highly intact RNA. RNA was purified from Jurkat cells using the RNeasy Mini Kit. The purified RNA was analyzed on the QIAxcel® system (ratio of 28S to 18S rRNA: 1.55) and Diagilent 2100 Bioanalyzer (ratio of 28S to 18S rRNA: 1.7). A high RNA integrity number (RIN) of 9.6 was obtained, indicating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

## Ordering Information

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

<sup>\*</sup> Automatable on the QIAcube. Find out more at <a href="www.qiagen.com/goto/QIAcube">www.qiagen.com/goto/QIAcube</a>

## QIAGEN products for sample disruption

## 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.

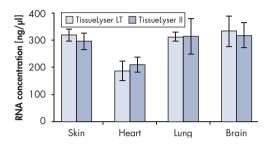


Figure 16. Effective tissue disruption. Various rat tissues were disrupted using the Tissuelyser LT or Tissuelyser II. RNA was purified from 20 mg samples on the QIAcube® using the RNeasy Fibrous Tissue Mini Kit (brain). RNA was eluted in a volume of 50  $\mu$ l, and concentration was determined using 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	<u>79654</u>
<u>TissueRuptor</u> ®	For disruption of individual samples	9001271
TissueRuptor Disposable Probes (25)	Disposable probes for use with the TissueRuptor	990890
<u>TissueLyser LT</u>	For disruption of up to 12 samples	85600
TissueLyser LT Adapter, 12-tube	For purification of RNA from cells in 96-well format	<u>69980</u>
<u>TissueLyser II</u>	For disruption of up to 48 or 192 samples	<u>85300</u>
<u>TissueLyser Adapter Set 2 x 24</u>	Adaptor set for use with the Tissuelyser II; holds 48 tubes	<u>69982</u>

## QIAGEN products for RNA stabilization

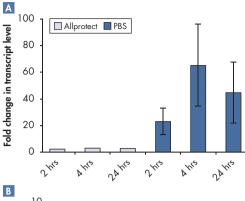
### What is RNA stabilization?

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.



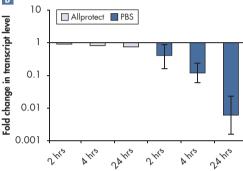


Figure 17. Effective RNA stabilization. Rat tissues were stored at 25°C for 2–24 hours in Allprotect Reagent or PBS prior to real-time RT-PCR analysis. A Rat lung tissue was analyzed for c-fos expression. B Rat intestine tissue was analyzed for Madh7 expression. Transcript levels relative to those in 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	<u>76405</u>
AllPrep® DNA/RNA Mini Kit (50)	For simultaneous purification of DNA and RNA	80204
RNA <i>later</i> ® RNA Stabilization Reagent (250 ml)	For stabilization of RNA in tissues	<u>76106</u>
RNeasy Protect Mini Kit (50)	For stabilization of RNA in tissues and RNA purification	<u>74124</u>
RNAprotect® Cell Reagent (250 ml)	For stabilization of RNA in cells	<u>76526</u>
RNAprotect Animal Blood Tubes (50 x 100 µl)	For collection of 100 µl animal blood with RNA stabilization	<u>76544</u>
RNeasy Protect Animal Blood Kit (50)	For purification of RNA from blood collected in RNAprotect Animal Blood Tubes	<u>73224</u>
miRNeasy Protect Animal Blood Kit (50)	For purification of RNA, including miRNA, from blood collected in RNAprotect Animal Blood Tubes	<u>217304</u>

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## RT<sup>2</sup> Profiler PCR Array quick index

Research area	Apoptosis Research	Biomarker Research	Cancer Research	Cell Cycle Research
RT <sup>2</sup> Profiler PCR Array listing	Apoptosis	Alzheimer's Disease	Angiogenesis	Apoptosis
For catalog numbers, see page 2 or visit: www.SABiosciences.com/ArrayList.php  Try RT² Profiler PCR Arrays	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
	NFkB 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
	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
	Ubiquitination Pathway	T-cell and B-cell Activation	Tumor Suppressor Genes	Transcription Factors
Risk-free starter pack offer details:		, surrainon		
www.SABiosciences.com/PCRguide.php	Unfolded Protein Response	Th1-Th2-Th3	WNT Signaling Pathway	Ubiquitination Pathway

RT<sup>2</sup> Profiler PCR Array free data analysis tool, see page 10. <u>http://sabiosciences.com/pcrarraydataanalysis.php</u>

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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	NFkB Signaling Pathway	Mesenchymal Stem Cell	NFĸB 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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