

Gene Expression Analysis You Can Count On
Signal Output True to the Original RNA



We bring high resolution to your valuable gene expression analysis experiments.

Roche Applied Science provides a broad gene expression product portfolio of reagents and systems optimized for accurate determination of even small differences in mRNA expression levels.

Obtain the full range of high resolution gene expression products from a manufacturer with over a decade of experience. Choose Roche Applied Science to accompany your research for the future.

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High Performance Essentials for the Gene Expression Workflow

Everything Depends on the Quality of the RNA (see pages 6–11)

Robust fluorescent gene expression signals require high-quality input RNA. Whether you use manual, such as the **High Pure RNA Isolation Kit**, or automated RNA purification, such as the **Magna Pure LC 2.0 Instrument** with handsfree RT-PCR set up, all of our reagent kits and instruments preserve the RNA signal-generating power of the original sample.

cDNA Synthesis Preserves Your RNA Transcripts (see pages 12–15)

First Strand Transcriptor reverse transcriptase systems with **Protector RNase Inhibitor**, permanently and precisely, preserve the original RNA relationships for downstream gene expression (and qPCR) studies. For trace amounts of RNA, the new **LightCycler® RNA Pre-Amplification Kit** produces up to 1,500-fold enriched amplified cDNA, still preserving the original transcript proportions.

Combine the Universal ProbeLibrary with Real-time PCR and Microarrays (see pages 16–20)

To detect a gene of choice, simply use **ProbeFinder Software** to select one of 165 fluorescent **Universal ProbeLibrary** probes to quantify your specific RNA transcript. **RealTime ready Focus Panels** containing qPCR assays targeting a broad range of selected genes corresponding to specific functional pathways are both cost-effective and convenient. The **Roche NimbleGen** product line of high-density DNA microarrays provides the complete solution for gene expression profiling analysis. For real-time PCR, **LightCycler® 480 Instruments** produce exquisite fluorescent signals from 96-well or 384-well plates using state-of-the-art one- or two-step reverse transcription, amplification systems.

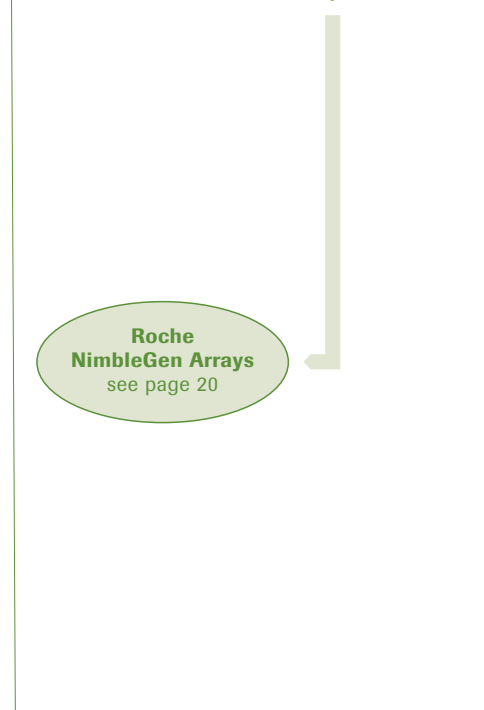
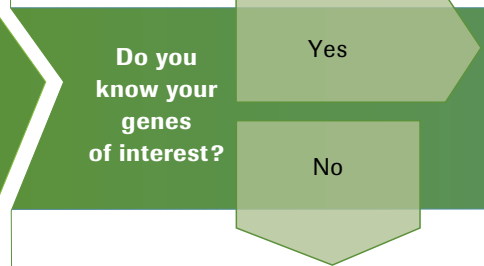
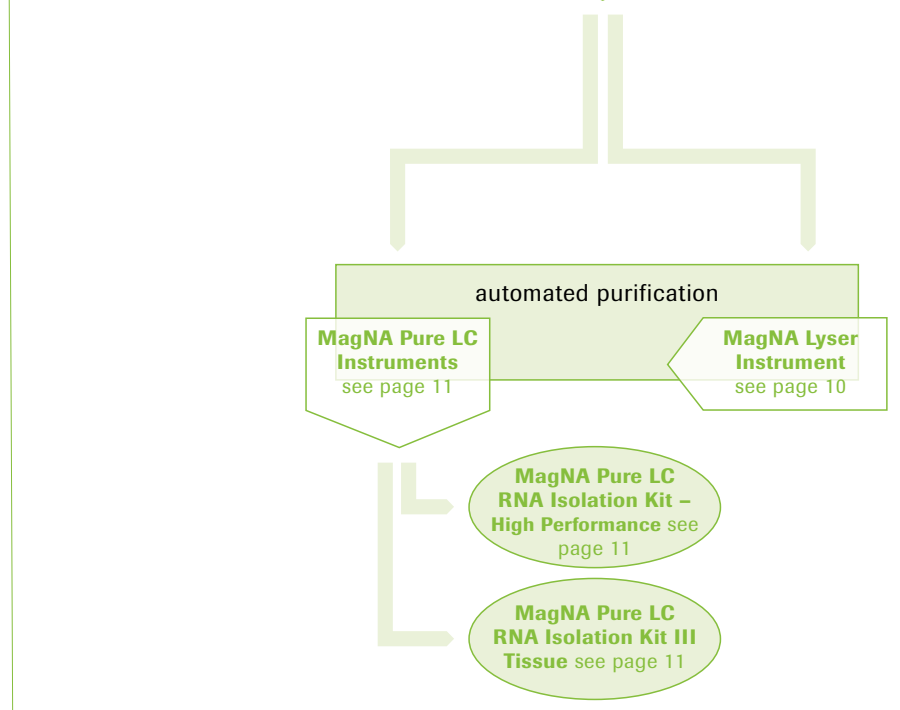
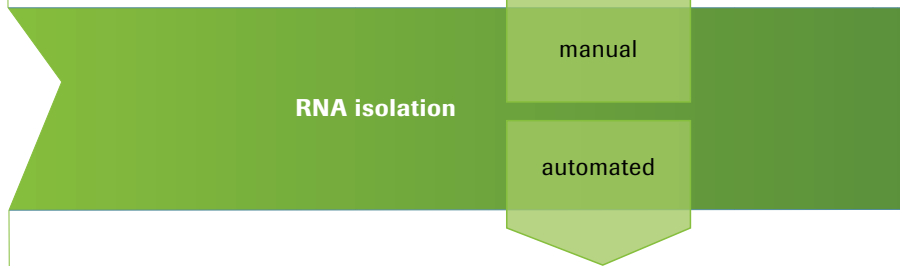
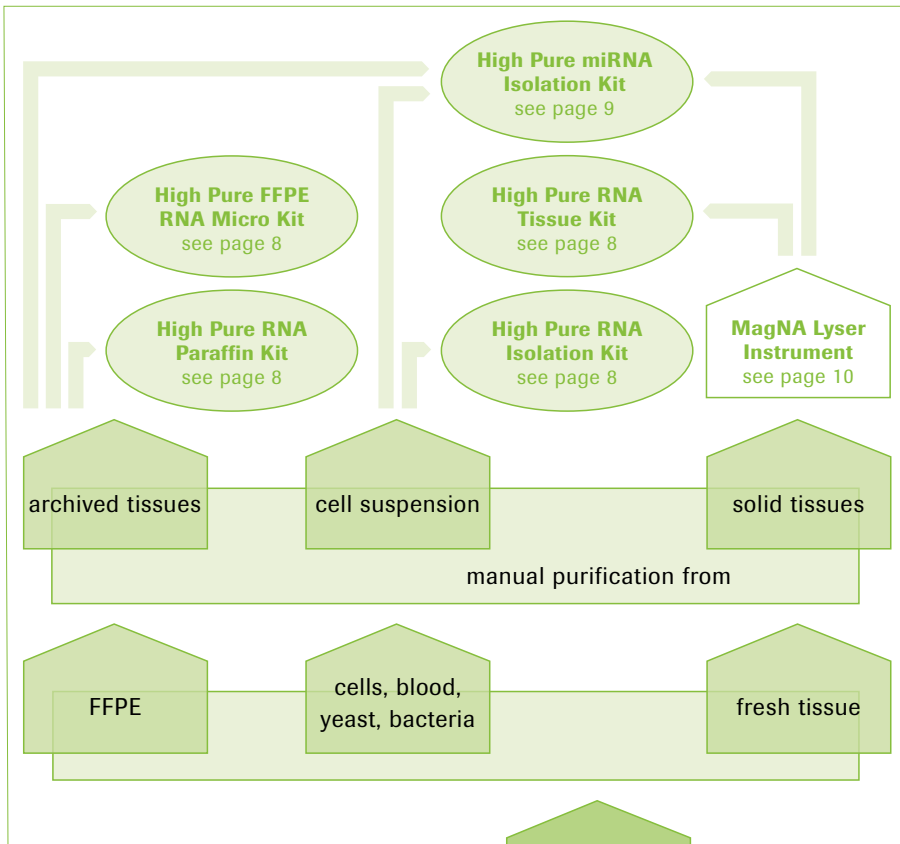
How to use the Product Selection Guide (see pages 4–5)

Use the Gene Expression Analysis Selection Guide on the next two pages as an overview of the workflow using our products. Identify the product combinations required by your application. Find and read the thumbnail descriptions of typical results using the page numbers provided.



RNA Isolation

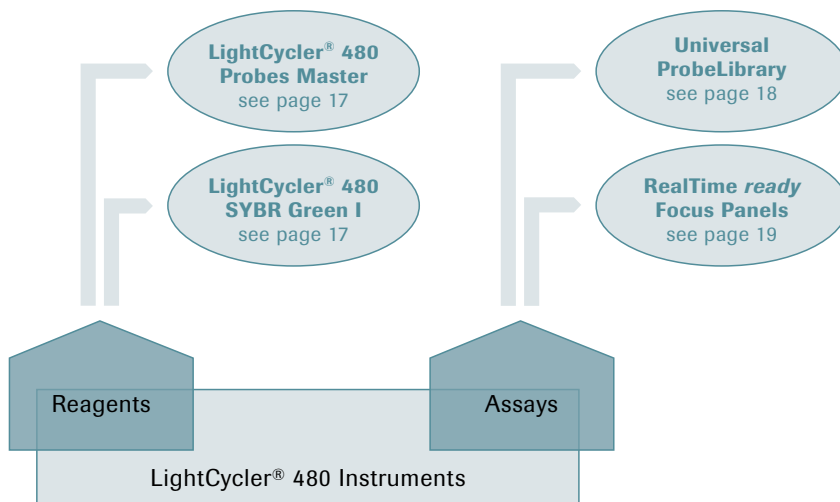
cDNA



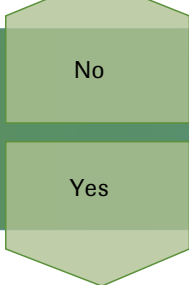
Synthesis

Real-Time PCR

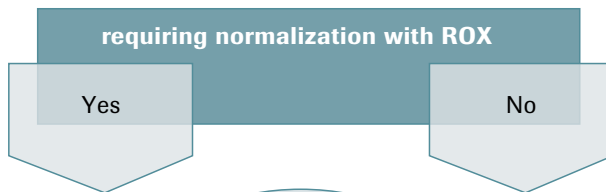
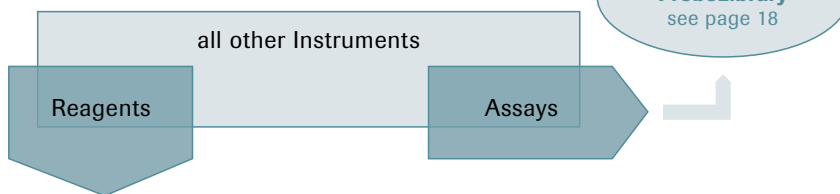
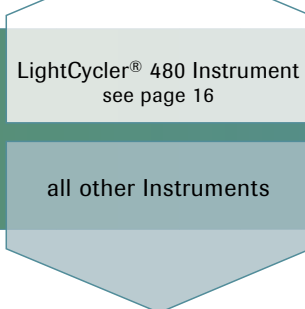
- Transcriptor Reverse Transcriptase see page 14
- Transcriptor First Strand cDNA Synthesis Kit see page 13



Do you require RNA pre-amplification?



Real-time PCR on



- LightCycler® 480 Pre-Amplification Kit see page 13



RNA Isolation

*Gene expression breakthroughs
require high-quality RNA*

RNA
Isolation

Roche Applied Science offers High Pure series kits for manual isolation of RNA, and MagNA Pure LC 2.0 kits for automated purification of RNA, from all possible sample types, from bacteria to human formalin-fixed, paraffin-embedded (FFPE) tissue sections.

The fluorescent signal achieved after cDNA synthesis and real-time PCR, is a reflection of the quality of the input RNA. Carefully crafted molecular reagents manufactured to the highest standard are the prerequisite for discoveries in gene expression analysis.

- **Use the highest quality RNA possible** for sensitive and accurate gene expression analysis.
- **Achieve your goals using our High Pure products for the manual isolation of high-quality RNA** from a wide variety of sample material.
- **Automate RNA isolation using the MagNA Pure LC 2.0 robotic workstation** when throughput and reproducibility are the goal.

Manual RNA Isolation

High Pure nucleic acid isolation kits – flexibility unlimited

Target Nucleic Acid	Origin/Source	Products
Total RNA	Mammalian cultured cells	High Pure RNA Isolation Kit
	Blood cells	
	Homogenized tissues	
	Bacteria or Yeast cells	
	Solid tissues	High Pure RNA Tissue Kit
Micro RNA	Formalin-fixed, paraffin-embedded (FFPE) tissue sections	High Pure FFPE RNA Micro Kit High Pure RNA Paraffin Kit
	All above mentioned sample types	High Pure miRNA Purification Kit

▲ **Table 1: RNA isolation kits best suited to your sample type**

Silica adsorption of total RNA in finely-engineered spin columns including an on-column DNA digestion with DNase I, is the perfect way to begin your low to medium throughput workflow for gene expression analysis.

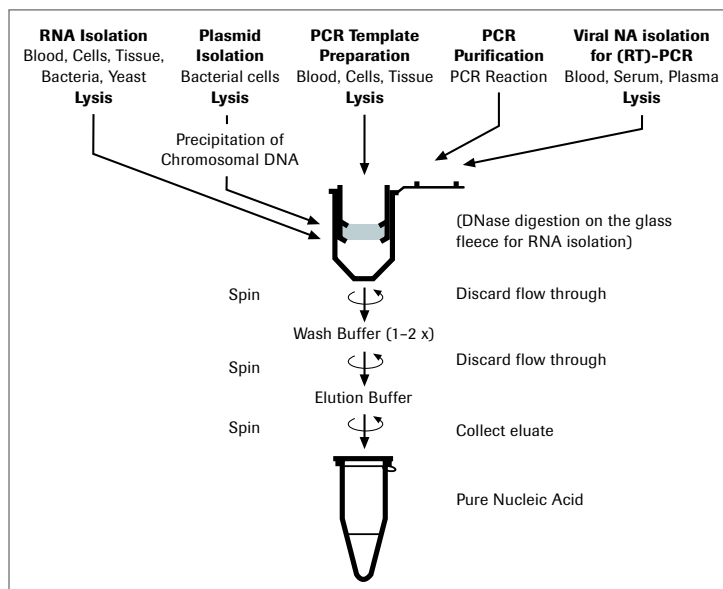
All these cost-effective, time-saving kits achieve excellent recovery, showing optimal sensitivity and linearity (scalability when using increasing amounts of starting material) in qRT-PCR and other gene expression assays.



▲ **Figure 1: High Pure Micro Spin Column** for > 10 µl elution volume.



▲ **Figure 2: High Pure Mini Spin Column** for > 50 µl elution volume.



▲ **Figure 3: High Pure Spin Column technology.**

Manual RNA Isolation

High Pure nucleic acid isolation kits – flexibility unlimited

High Pure RNA Isolation Kit

High Pure RNA Tissue Kit

For isolation of intact total RNA, we offer two kits depending on the sample material used. For mammalian cells, whole blood and a variety of microorganisms, choose the High Pure RNA Isolation Kit. For solid tissue, use the High Pure RNA Tissue Kit.

- Profit from the High Pure kit's outstanding integrated DNase-treatment step.** Degrade genomic DNA that would co-purify and reduce the quality of downstream steps.
- Obtain the high RNA yields required for all gene expression analysis applications.** Produce high yields of total RNA from a vast variety of sample types (Tables 2 and 3 below).
- Minimize RNA loss with the convenient spin column approach.** Remove contaminants in minutes and avoid carry-over contamination for optimal performance in qRT-PCR.

High Pure RNA Isolation Kit

Starting Material	Sample Size	RNA Yield
Cultured cells (K-562)	10 ⁸ cells	Up to 15 µg
Human whole blood	200 µl	10 RT-PCR
Yeast (<i>S. cerevisiae</i>)	10 ⁸ cells	Up to 20 µg
Bacteria (<i>E. coli</i>)	10 ⁹ cells	Up to 50 µg
Bacteria (<i>B. subtilis</i>)	10 ⁹ cells	Up to 35 µg

▲ Table 2: RNA yields from selected starting materials

High Pure RNA Tissue Kit

Starting Material	Average RNA Yield (µg/mg)
Liver	0.5 - 2.8 µg
Kidney	0.5 - 1.0 µg
Spleen	0.5 - 3.0 µg
Lung	0.3 - 0.5 µg
Heart	0.3 µg

▲ Table 3: RNA yields from selected starting materials

Product	Cat. No.	Pack Size
High Pure RNA Isolation Kit	11 828 665 001	50 purifications
High Pure RNA Tissue Kit	12 033 674 001	50 purifications

High Pure FFPE RNA Micro Kit

High Pure RNA Paraffin Kit

To maximize your yields of total RNA from different types of challenging formalin-fixed, paraffin-embedded (FFPE) tissue samples we provide the following two solutions:

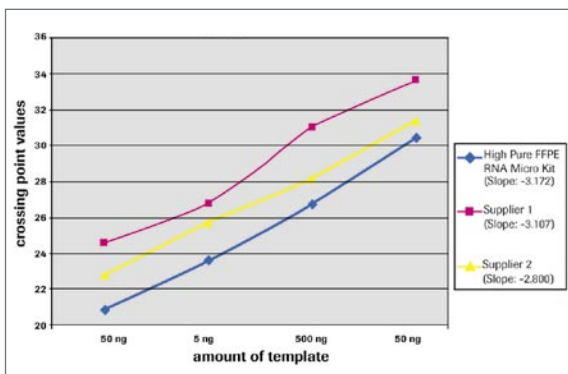
The High Pure FFPE RNA Micro Kit and the High Pure RNA Paraffin Kit can be used for FFPE sections from a variety of sources, including colon, breast, liver, kidney, or spleen of mammalian human research samples.

The High Pure FFPE RNA Micro Kit

- **Adds momentum to your research.** Use a simple, fast (half-day) protocol and innovative column design to isolate high-quality RNA.
- **Concentrates your RNA.** Obtain total RNA from 1 to 10 µm-thick tissue sections in a final eluate as low as 10 µl.

The High Pure RNA Paraffin Kit

- **Maximizes your yield.** Use a more stringent overnight protocol to purify total RNA from pooled FFPE (10 to 20 µm), and also fresh-frozen tissue sections in a final eluate of 50 µl.



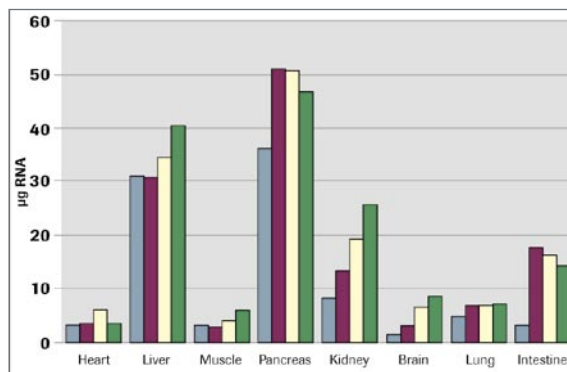
▲ **Figure 4: RNA templates isolated with the High Pure FFPE RNA Micro Kit produces earlier crossing points compared to competitor RNA purification kits.** Data shown above were produced using a two-step, SYBR Green I, qRT-PCR assay of a 3 log dilution for different RNA amounts (50 ng, 5 ng, 500 pg, 50 pg) using the LightCycler® Instrument to detect β 2 microtubulin transcripts.

Product	Cat. No.	Pack Size
High Pure FFPE RNA Micro Kit	04 823 125 001	Up to 50 isolations
High Pure RNA Paraffin Kit	03 270 289 001	Up to 100 isolations

High Pure miRNA Isolation Kit

Choose the one- or two-column protocol provided when using the High Pure miRNA Isolation Kit, to easily and quickly isolate either (1) high-purity total RNA that includes micro RNA, or (2) highly enriched miRNA, respectively.

- **Purify miRNA without the need for hazardous organic solvents.** Isolate high-quality RNAs without the use of toxic phenol/chloroform.
- **Obtain high yields from a simple, efficient protocol.** Recover high yields of purified total RNA and miRNA through a straightforward protocol.
- **Choose one flexible kit for all your miRNA purifications.** Use the same versatile kit to purify small RNAs from a variety of sample types, including both fresh and FFPE tissues.



▲ **Figure 5: Yield comparison of miRNA isolated from different tissues with High Pure miRNA Isolation Kit.**
 ■ FFPE tissue / RNA Purification Kit from Supplier 1;
 ■ FFPE tissue / RNA Purification Kit from Supplier 2;
 ■ FFPE tissue / RNA High Pure miRNA Isolation Kit from Roche Applied Science;
 ■ Fresh-frozen tissue extracted with phenol/chloroform.

Product	Cat. No.	Pack Size
High Pure miRNA Isolation Kit	05 080 576 001	Up to 50 miRNA isolations

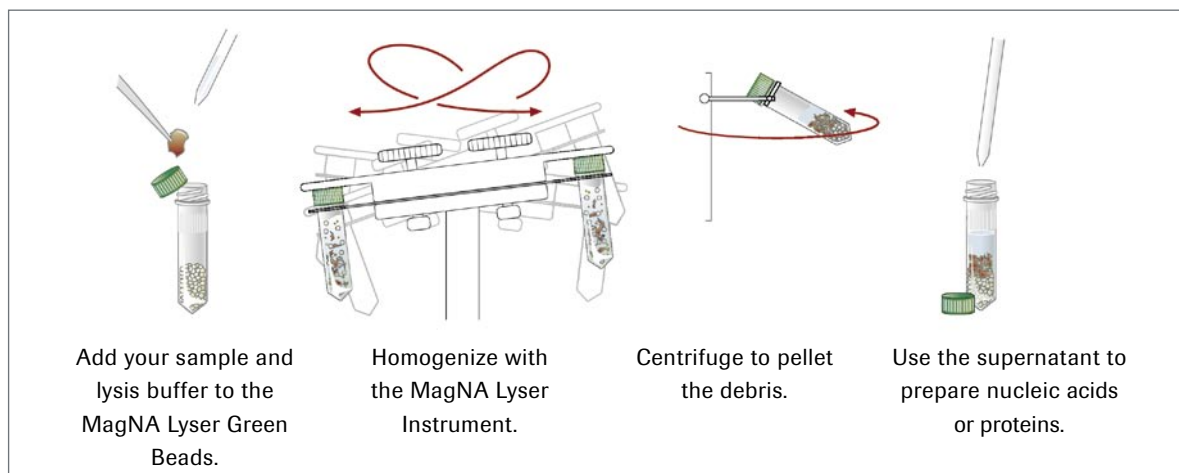
Sample Homogenization

Release the RNA from difficult samples

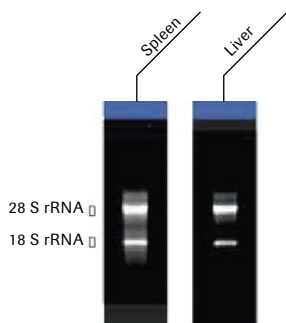
MagNA Lyser Instrument

This benchtop instrument with a small footprint can disrupt and homogenize up to 16 samples of a wide variety of sample materials in seconds. Dedicated cooling blocks ensure sample integrity, and facilitate convenient handling.

Prefilled disposable vials contain the **MagNA Lyser Green Beads** that collide to produce the necessary mechanical energy to disrupt a wide variety of cells. Resulting supernatants containing nucleic acids and proteins are ideally suited for subsequent purification of RNA for high performance quantitative gene expression analysis.



▲ **Figure 6: The MagNA Lyser tissue homogenization principle.**



◀ **Figure 7: Gel electrophoresis from total RNA isolated from tissue homogenized with the MagNA Lyser Instrument, using the MagNA Pure RNA Kit III (Tissue) on the MagNA Pure LC Instrument.**

Product	Cat. No.	Pack Size
MagNA Lyser Instrument	03 358 968 001	1 instrument (110V)
MagNA Lyser Instrument	03 358 976 001	1 instrument (220V)
MagNA Lyser Green Beads	03 358 941 001	100 tubes prefilled with 1.4 mm diameter ceramic beads

Automated RNA Purification

Technology meets performance



The MagNA Pure LC 2.0 Instrument

The MagNA Pure LC 2.0 Instrument provides walk-away automation and proven RNA isolation kits, freeing your hands for the other important steps in the gene expression analysis workflow. RNA for your gene expression studies is purified using dedicated high-quality reagents and kits, featuring user-friendly software protocols customized to your sample requirements.

This robotic workstation for nucleic acid purification uses proven magnetic bead technology, thus eliminating the need for filtration, centrifugation and organic solvents.

- **Isolate high-purity nucleic acids.** Use proven reagent kits combined with optimized purification protocols.
- **Take advantage of flexible protocols** and obtain high RNA yields from mammalian whole blood, including PAXgene-stabilized RNA, from cultured cells, as well as from frozen tissues and FFPE sections.
- **Enjoy consistent purification** and reproducible results driven by proven magnetic bead technology.

Product	Cat. No.	Pack Size
MagNA Pure LC 2.0 Instrument	05 197 686 001	1 instrument plus accessories

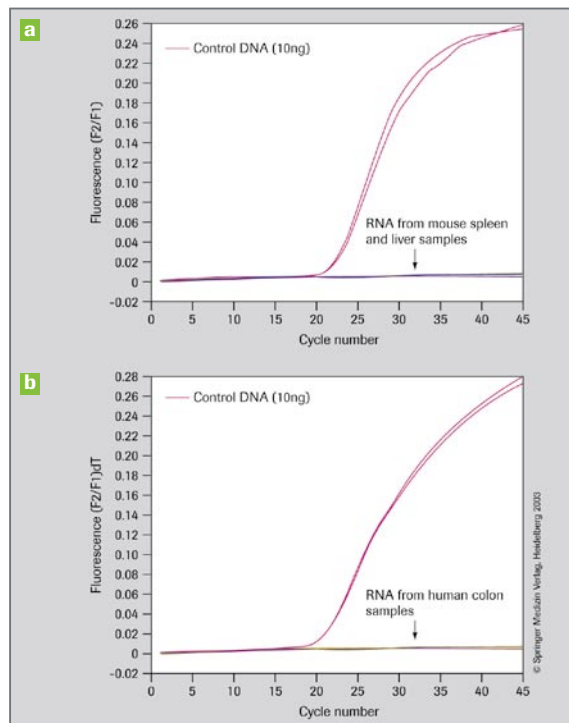
MagNA Pure LC RNA Isolation Kit – High Performance

Isolate high-purity total RNA from mammalian whole blood, blood cells, or cultured cells.

MagNA Pure LC RNA Isolation Kit III – Tissue

Extract RNA from 1 to 10 mg fresh-frozen tissues, 5 to 10 μ m-thick formalin-fixed, paraffin-embedded (FFPE) tissue sections, and from samples pretreated with RNAlater (Ambion).

Product	Cat. No.	Pack Size
MagNA Pure LC RNA Isolation Kit – High Performance	03 542 394 001	Up to 192 purifications
MagNA Pure LC RNA Isolation Kit III (Tissue)	03 330 591 001	Up to 192 purifications



▲ **Figure 8: LightCycler® RT-PCR analysis of RNA isolated from mouse (a) and human tissue (b). No DNA was detected in the sample.**

cDNA Synthesis

*Accurate gene expression levels
by unbiased two-step qRT-PCR*

Choose the **Transcriptor First Strand cDNA Synthesis Kit**, or the appropriate single enzymes, to obtain more sensitive two-step qRT-PCR results on any real-time PCR instrument. Use isothermal amplification when minimal quantities of RNA are available. Save your RNA from degradation with the **Protector RNase Inhibitor**.

- **Carry out highly efficient, unbiased transcription from mRNA to cDNA**, from both high and low abundant mRNA simultaneously.
- **Obtain high resolution signals in the subsequent real-time PCR analysis** with reagents optimized for high fidelity signal dynamics.
- **Pre-amplify RNA from small samples**, and experience 1500-fold enrichment using the LightCycler® RNA Pre-Amplification Kit.

Application	Products
Two-step RT-PCR	Transcriptor First Strand cDNA Synthesis Kit*
	Transcriptor Reverse Transcriptase
Preamplified cDNA synthesis	LightCycler® RNA Pre-Amplification Kit
Protection of RNA	Protector RNase Inhibitor

* For either sequencing and cloning applications, analysis of splicing, or working with Universal ProbeLibrary probes, requiring the highest possible accuracy, choose the **Transcriptor High Fidelity cDNA Synthesis Kit**.

Two-Step Reverse Transcription

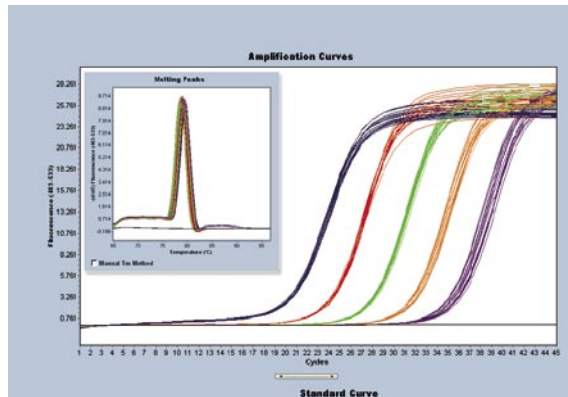
Increase the power and sensitivity of cDNA synthesis

Transcriptor First Strand cDNA Synthesis Kit

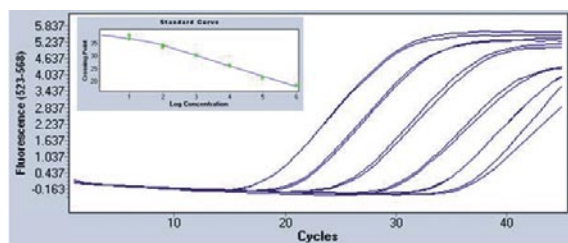
Improve gene-expression studies by using the Transcriptor First Strand cDNA Synthesis Kit to reverse transcribe RNA without distorting gene expression levels; then amplify targets in the following qPCR.

The Transcriptor First Strand cDNA Synthesis Kit contains all components necessary for a reverse transcription reaction, including the unique anchored-oligo (dT)₁₈ primer, Protector RNase Inhibitor, and control elements (control RNA and control PBGD primer mix).

- Increase sensitivity in quantitative RT-PCR** and simultaneously reverse transcribe rare and abundant RNA – without distorting gene expression levels. Generate a representative pool of cDNA for subsequent qPCR. Profit from optimally aligned products for your workflow (Figure 9 and 10).
- Achieve accurate linear quantification over at least a 10⁸-fold range of input RNA.** Analyze genes with very low or extremely high expression levels.
- Simplify results interpretation.** Produce qPCR curves that are well shaped, exhibit a high fluorescence intensity, and maintain identical distances between RNA dilutions.
- Overcome challenging secondary structures.** Reverse transcribe at temperatures up to 55°C, and power through difficult templates with high GC content.
- Produce large quantities of full-length cDNA.** Be confident and use a reverse transcriptase that generates very long cDNAs, up to 14 kb.



▲ **Figure 9: Quantification of the h-HPRT gene with the Transcriptor First Strand cDNA Synthesis Kit and the LightCycler® 480 Instrument.** Using the Transcriptor First Strand cDNA Synthesis Kit, total RNA was reverse transcribed by random hexamer priming; 100 ng/reaction to 10 pg/reaction of the transcribed RNA was used in a subsequent PCR. Using the LightCycler® 480 Instrument, eighteen-fold repeats of h-HPRT can be detected with high reproducibility (delta Cp ranging from 0.29 to 1.06, depending on the dilution).



▲ **Figure 10: Profit from workflow-proven products.** Total RNA of K562 cells was purified by High Pure RNA Isolation Kit. 1 pg to 1 µg aliquots were reverse transcribed with Transcriptor First Strand cDNA Synthesis Kit. The following PCR was performed on the LightCycler® 480 System in duplicate with the LightCycler® 480 Probe Master and an UPL probe for β-actin. Roche products for gene expression workflow permit simultaneous detection of rare and abundant transcripts.

Product	Cat. No.	Pack Size
Transcriptor	04 379 012 001	Up to 50 reactions
First Strand	04 896 866 001	Up to 100 reactions
cDNA	04 897 030 001	Up to 200 reactions
Synthesis Kit		

Two-Step Reverse Transcription

Increase the power and sensitivity of cDNA synthesis

Transcriptor Reverse Transcriptase

Choose the recombinant Transcriptor Reverse Transcriptase single reagent for customized applications, when the additional components of the other RT-PCR components will be purchased separately. mRNA, total RNA, viral RNA, or *in vitro*-transcribed RNA from a variety of sources can be used. Transcriptor Reverse Transcriptase is a multi-purpose enzyme tested in a variety of different applications. Use it for two-step RT-PCR, labeling of cDNA, retrieval and cloning of the 5'- and 3'-termini of mRNA by RACE, or generation of cDNA libraries. High sensitivity, in combination with high thermostability, make this enzyme ideal for RT-PCR.

- **Achieve high sensitivity in two-step RT-PCR in all real-time PCR instruments (e.g., the LightCycler® Instruments).**
- **Reverse transcribe difficult GC-rich RNA templates with an enzyme that is stable at elevated temperatures.**
- **Efficiently label cDNA with Cy3-, Cy5-, DIG-, biotin-, or aminoallyl-labeled nucleotides during cDNA synthesis.**
- **Produce large quantities up to 14 kb full-length cDNA.**

Product	Cat. No.	Pack Size
Transcriptor Reverse Transcriptase	03 531 317 001	250 U (25 reactions)
	03 531 295 001	500 U (50 reactions)
	03 531 287 001	2000 U (200 reactions)

Protector RNase Inhibitor

Inhibit a wide spectrum of RNases with Protector RNase Inhibitor, and protect your RNA samples from RNases, and thereby from degradation. This thermostable inhibitor is fully active during cDNA synthesis (e.g., with thermostable reverse transcriptases like Transcriptor Reverse Transcriptase). Use the versatile Protector RNase Inhibitor in any application where RNases are a potential problem.

- **Rely on an active RNase Inhibitor during cDNA synthesis** that is active up to 60°C.
- **Use it in combination with different enzymes and buffers** between pH 5.0 – 9.0.
- **Eliminate interference, even at high concentrations.** Protector RNase Inhibitor does not inhibit or otherwise interfere with enzyme systems used to analyze RNA.

Each batch is function-tested using techniques like quantitative RT-PCR to ensure the absence of endonucleases, ribonucleases, or nicking activities.

Type of RNase	Amount Inhibited
RNase A	Up to 1 ng
RNase B	Up to 160 ng
RNase T2	Up to 0.03 U/μl reaction volume

Table 4: Inhibition of different types of RNases by Protector RNase Inhibitor.

Product	Cat. No.	Pack Size
Protector	03 335 399 001	2000 U
RNase Inhibitor	03 335 402 001	10000 U

Two-Step Reverse Transcription from Very Small Samples

More data from precious samples

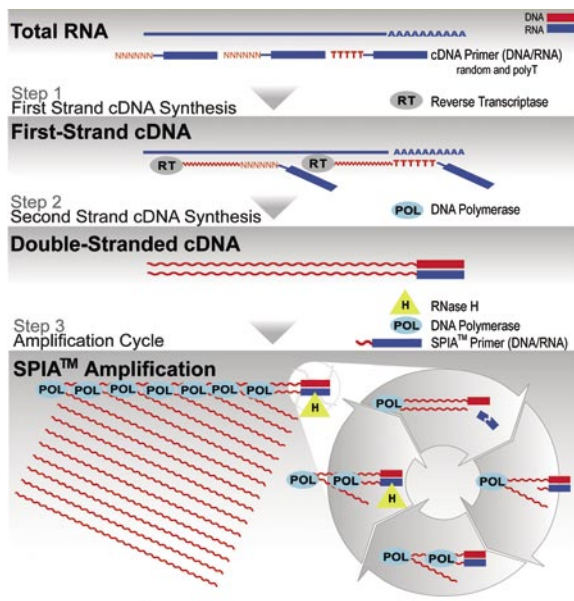
LightCycler® RNA Pre-Amplification Kit

There is growing interest in using gene expression research studies of clinical tissue or blood samples to identify new molecular targets, better molecular diagnostic tools, and biomarkers for drug efficacy.

- **Achieve fast and efficient PCR template preparation.** Up to 1,500- fold amplified cDNA in only four hours from 5 to 50 ng total RNA.
- **Maintain the relative transcript representation.** Keep the signature of your original RNA sample with the selective Ribo-SPIA® isothermal linear amplification method.
- **For direct use.** Use the amplified cDNA with standard PCR reaction.

The LightCycler® RNA Pre-Amplification Kit provides a fast and simple method for preparing high yields of amplified cDNA from nanogram amounts of total RNA for subsequent gene expression analysis with qPCR. The Ribo-SPIA®* linear isothermal amplification technology used in this kit reduces contamination by amplifying only original copies of the starting mRNA. The amplified product is single-stranded cDNA in the antisense direction of the RNA starting material; the cDNA product is ready for qPCR and does not require purification.

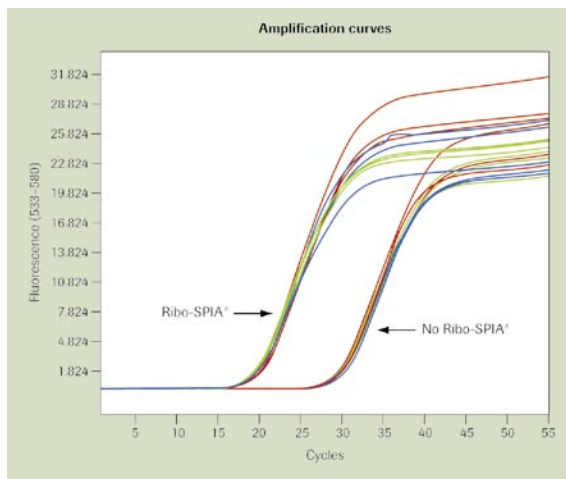
*RNA amplification by NUGEN Technologies, Inc.



▲ **Figure 11: Ribo-SPIA® whole transcriptome amplification process.**

Ribo-SPIA® technology is a three-step process that generates amplified cDNA from as little as 5 ng of total RNA.

Product	Cat. No.	Pack Size
LightCycler® RNA Pre-Amplification Kit	05 190 894 001	For up to 32 reactions



	Cp after pre-amplification	Cp without pre-amplification
Total RNA, source 1 (n=3)	20.47	30.22
Total RNA, source 2 (n=3)	19.95	30.47
Total RNA, source 3 (n=3)	19.79	30.23

▲ **Figure 12: The fluorescence exponential curves produced using the real-time LightCycler® 480 PCR System show a significant signal increase when using the LightCycler® RNA Pre-Amplification Kit.**

On average, crossing points (Cp) appear 10 cycles earlier when using the LightCycler® Pre-Amplification Kit.

Automated Real-Time PCR

Fast-cycling solution for accurate gene expression analysis

LightCycler® 480 Instrument

The LightCycler® Instrument is a fully integrated multi well-plate based real-time PCR platform for highly accurate qualitative and quantitative detection of nucleic acids. Providing novel ways to combine speed and accuracy without compromises, the LightCycler® 480 Real-Time PCR System ideally meets the needs of relative quantification studies, *e.g.*, for validation of array data.

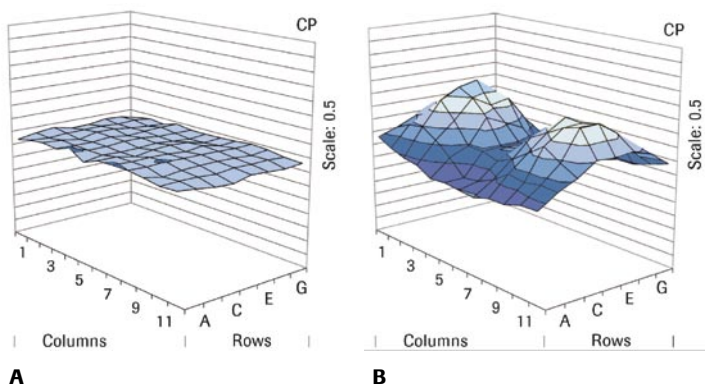
Unsurpassed accuracy and precision in less than 40 minutes

- **Experience novel high-speed thermal cycling and data capture technology** for unparalleled temperature homogeneity and assay reproducibility.
- **Flexibly switch between 96- or 384-well plates in clear or white formats**, exchanging thermal block cyclers in just minutes without recalibrating.



The LightCycler® 480 System workflow exemplifies versatility and sensitivity

- **Analyze true raw data**, breaking free of the need to use passive reference dyes, or normalization plates, to get accurate results.
- **Conveniently analyze real-time PCR data** using either basic or advanced proprietary algorithms for relative quantification, including multiple plate statistical analysis.
- **Use the LightCycler® 480 Systems networking capabilities** to create a Laboratory Information Management System (LIMS) workflow that includes robotic pipetting and plate loading.



▲ **Figure 13: Precise engineering of state-of-the-art components is essential for accuracy, sensitivity and reproducibility during real-time PCR of 96-well and 384-well plates.** Crossing point (Cp) homogeneity is very high across a 96-well plate: for the LightCycler® 480 Instrument (shown in A), but much lower for another real-time PCR instrument (shown in B).

Product	Cat. No.	Pack Size
LightCycler® 480 Instrument	05 015 278 001	1 instrument (96-well version)
	05 015 243 001	1 instrument (384-well version)

LightCycler® 480 Reagents for Gene Expression Analysis

LightCycler® 480 Reagents are ready-to-use, 2x concentrated, hot start reaction mixtures for PCR based on FastStart Taq DNA Polymerase. The master mixtures are optimized for a fixed MgCl₂ concentration, which works with nearly all primer combinations.

Increase the speed and sensitivity of your gene expression analysis in both probe-based and SYBR Green I assays.

LightCycler® 480 Masters are automation-ready due to their stability at room temperature for 24 hours and at +2 to +8°C for 4 weeks without loss of efficiency.

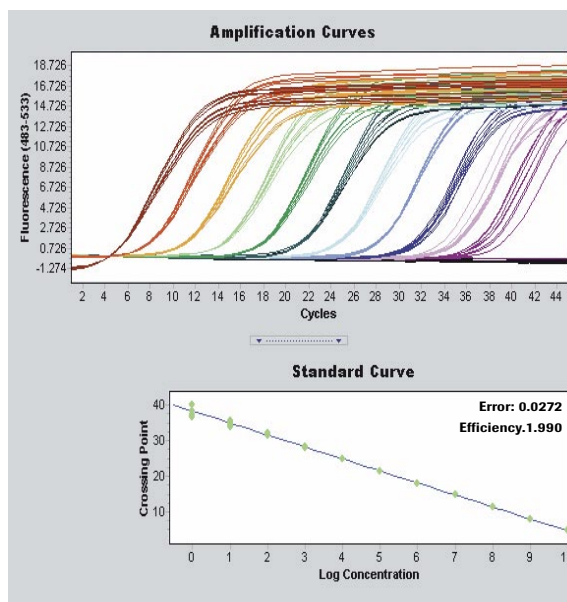
The mixtures can be used with all types of genomic or cDNA, and are ideally suited for high-throughput applications in 96- or 384-well plates.

Use these reagents to:

- **Minimize pipetting steps and contamination risk.**
- **Perform sensitive and specific quantitative PCR in clear or white plates.**
- **Eliminate time-consuming MgCl₂ titration.**

Establish new qPCR assays and get results quickly by combining LightCycler® 480 Probes Master with **Universal ProbeLibrary probes** or **RealTime ready Focus Panels**.

Product	Cat. No.	Pack Size
LightCycler® 480 Probes Master	04 707 494 001	5 x 1 ml (500 x 20 µl reactions)
	04 887 301 001	10 x 5 ml (5000 x 20 µl reactions)



▲ **Figure 14: Linear range of the LightCycler® 480 System.**

Serial 1:10 dilutions (nine replicates each) of a plasmid DNA sequence were amplified with the LightCycler® 480 Probes Master and detected with a UPL probe. The PCR result shows a log-linear relationship over a broad dynamic range (10 log-intervals) and highly reproducible Cp values for replicates of each dilution.

■ NTC	■ 10 ²	■ 10 ⁵	■ 10 ⁸
■ 1	■ 10 ³	■ 10 ⁶	■ 10 ⁹
■ 10	■ 10 ⁴	■ 10 ⁷	■ 10 ¹⁰

Product	Cat. No.	Pack Size
LightCycler® 480 SYBR Green I Master	04 707 516 001	5 x 1 ml (500 x 20 µl reactions)
	04 887 352 001	10 x 5 ml (5000 x 20 µl reactions)

Real-Time PCR Reagents and Assays

Proven solutions for design, amplification and detection

Universal ProbeLibrary

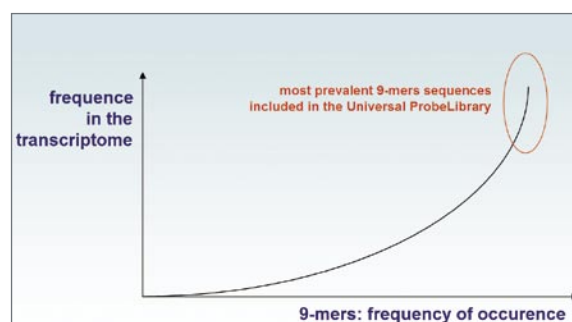
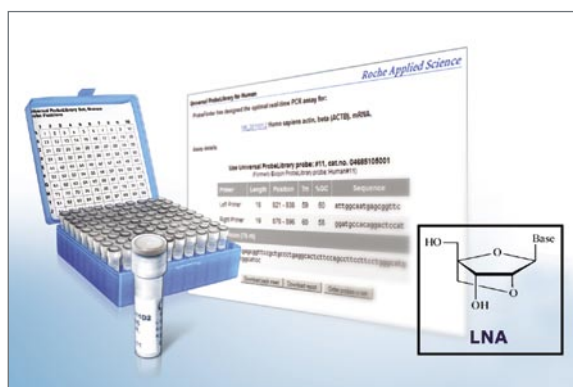
Perform millions of qPCR assays using only the 165 different proven hydrolysis probes found in the Universal ProbeLibrary (UPL). UPL probes are labeled with either FAM for target genes or LightCycler® 555 Yellow for reference genes at their 5' end, and they all have a signal-blocking quencher at their 3' end.

Being only 8 to 9 nucleotides in length, UPL probes contain Locked Nucleic Acids (LNA). LNAs are nucleic acid analogs with a locked ribose conformation, that enhances base stacking, thus increasing thermal stability. LNA-containing UPL probes discriminate single base mismatches under conditions not possible with other nucleic acids, thus easily providing the specificity your gene expression assays require.

Importantly, only 165 UPL probes are required to quantify virtually any transcript for a large number of organisms. Combined with the free and easy-to-use, web-accessible UPL Assay Design Center software tool (found at www.universalprobelibrary.com), dual-color relative quantification for specific target genes is now just a click away.

UPL probes are compatible with all real-time PCR instruments capable of detecting fluorescein, FITC, FAM, and/or SYBR Green I. Their application has produced many published high resolution results using the LightCycler® 480 and Carousel-Based Systems, ABI 7500 and ABI 7900HT, and other platforms. The 165 UPL probes covering the entire transcriptome of many different prokaryotic and eukaryotic organisms, are easily stored in your freezer, providing universal flexibility for real-time PCR assays at any time!

- **Increase the specificity and robustness of your gene expression assay** using transcript-specific, Universal ProbeLibrary (UPL) probes, instead of SYBR Green I.
- **Obtain virtually complete coverage of entire transcriptomes of multiple organisms** with only 165 different UPL probes.
- **Easily produce discriminating high-resolution dual-color gene expression experiments** by including the appropriate house-keeping-gene in your transcript-specific UPL assay.



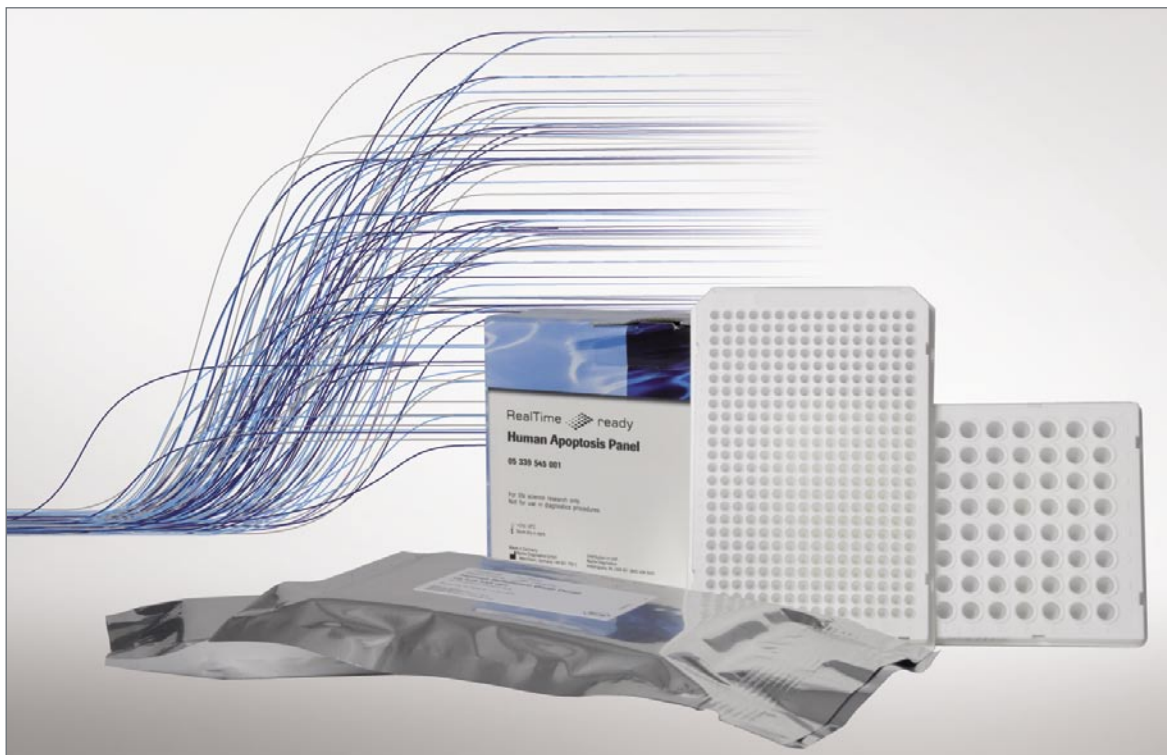
▲ **Figure 15: UPL probes detect highly prevalent 9-mer sequences.**

RealTime *ready* Focus Panels

The panels are composed of ready-to-use, pre-tested qPCR assays targeting a broad range of selected genes from specific pathways or functional groups. Conveniently supplied on LightCycler® 96- or 384-well plates, each assay contains target-specific primers and a Universal ProbeLibrary probe. All you need to add is master mix, water, and sample cDNA.

In addition to the pathway- or functional-group-specific targets, each plate also contains reverse transcription-specific controls and reference gene assays to facilitate data interpretation.

- **Get the complete picture of your expression levels.** Pre-designed panels with the relevant gene expression assays allow you to perform these complex analyses without the need of designing and combining the individual assays.
- **Choose from a selection of biological pathways and gene family panels** for immediate use on LightCycler® 480 instruments.
- **Functionally tested on reference RNA.** All PCR assays on RealTime *ready* Focus panels have been functionally tested on microarray quality control (MAQC) cDNA.



▲ Figure 16: RealTime *ready* Focus Panels

FastStart PCR Reagents

Combine accuracy and specificity with robust signal strength on ALL real-time PCR instruments

Select from four different FastStart PCR kits for hydrolysis probes or SYBR Green I, to maximize signal quality in qPCR gene expression studies, when using real-time platforms other than the LightCycler® Instrument.

These hot start master mixes, with or without ROX for normalization, consistently deliver sensitive, specific, and reproducible results.

Make the FastStart switch today to Roche Applied Science, for superb signal output from your real-time PCR!

- **Benefit** from Roche's experience and knowledge in producing real-time PCR master mixes for sensitive, high resolution analysis.
- **Use** universal reaction mixes optimized for real-time PCR no matter which instrument you are using.
- **Flexibly** choose between FastStart master kits for SYBR Green I or probe based assays, both available with or without ROX.
- **Combine** hydrolysis probes from the Universal ProbeLibrary with our FastStart Universal Probe Master to perform powerful gene expression studies on any real-time PCR platform.

Use this table to find a Master Mix for your instrument:

	Instruments requiring normalization with ROX Reference Dye <i>(e.g., ABI 7500 and 7900 Instruments)</i>	Instruments <i>not</i> requiring normalization with ROX Reference Dye
Detection with hydrolysis probes (e.g., Universal ProbeLibrary probes or TaqMan® probes)	FastStart Universal Probe Master (ROX)	FastStart TaqMan® Probe Master
Detection with sequence-independent dye SYBR Green I	FastStart Universal SYBR Green Master (ROX)	FastStart SYBR Green Master

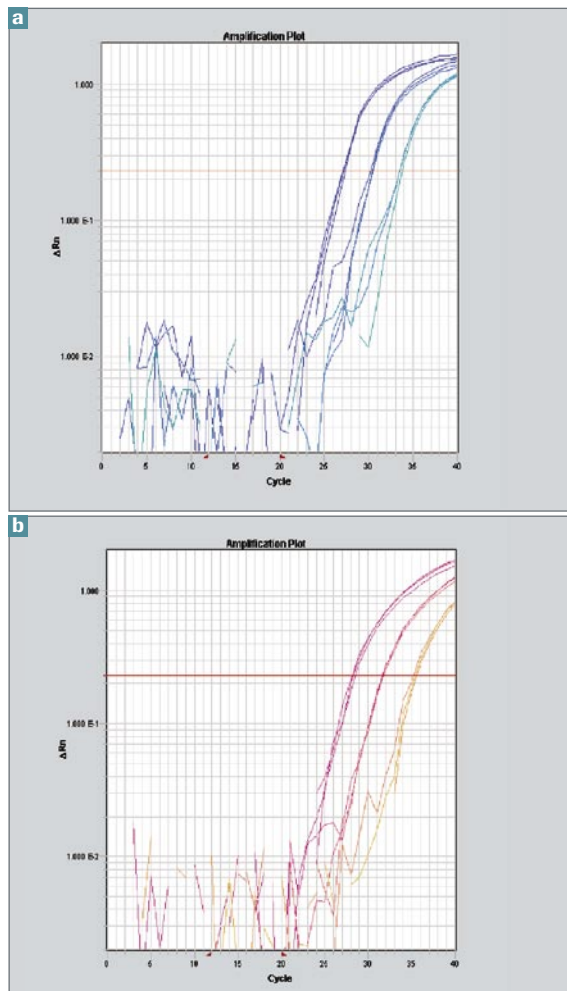
▲ **Table 5: Product Selection Guide.**

FastStart Universal Probe Master (Rox)

This master kit is a ready-to-use hot start PCR reagent mix that simplifies set up and produces optimal results in qPCR and two-step qRT-PCR results. With the appropriate PCR primers and an amplicon-specific hydrolysis probe, this kit enables highly sensitive detection and quantification of defined DNA sequences. Combine this mix with the Transcriptor First Strand cDNA Synthesis Kit to achieve the best possible fluorescent signal output in your two-step RT-PCR gene expression analysis workflow.

- **Benefit from this master mix's optimized buffer** enabling high-performance real-time PCR on all instruments that require reference dye normalization. FastStart Taq DNA Polymerase exceeds other enzymes in sensitivity, as shown by lower crossing point values after real-time PCR (see Figure 17).
- **Amplify and detect a broad range of DNA or cDNA targets.** Amplify fragments up to 500 bp long, including those that are GC- or AT-rich.
- **Use robotic pipetting stations for reaction set up.** Benefit from a master mix that is stable at room temperature during the extended reaction setup times required by robotic workstations.

Product	Cat. No.	Pack Size
FastStart Universal Probe Master (Rox)	04 913 949 001	2.5 ml (2 x 1.25 ml, for 100 reactions of 50 µl final volume)
	04 913 957 001	12.5 ml (10 x 1.25 ml, for 500 reactions of 50 µl final volume)
	04 914 058 001	50 ml (10 x 5 ml, for 2,000 reactions of 50 µl final volume)



▲ **Figure 17: Performance of FastStart Universal Probe Master on an ABI 7900.** The data shows a comparison of Roche FastStart Universal Probe Master (a) and a master mix from another supplier (b). The reactions utilized a hydrolysis probe specific for the globin gene along with a titration of human genomic DNA at concentrations of 50, 5, 0.5, and 0 ng/well. The FastStart Universal Probe Master data demonstrates earlier C_t -values and consistent, sensitive results.

FastStart PCR Reagents

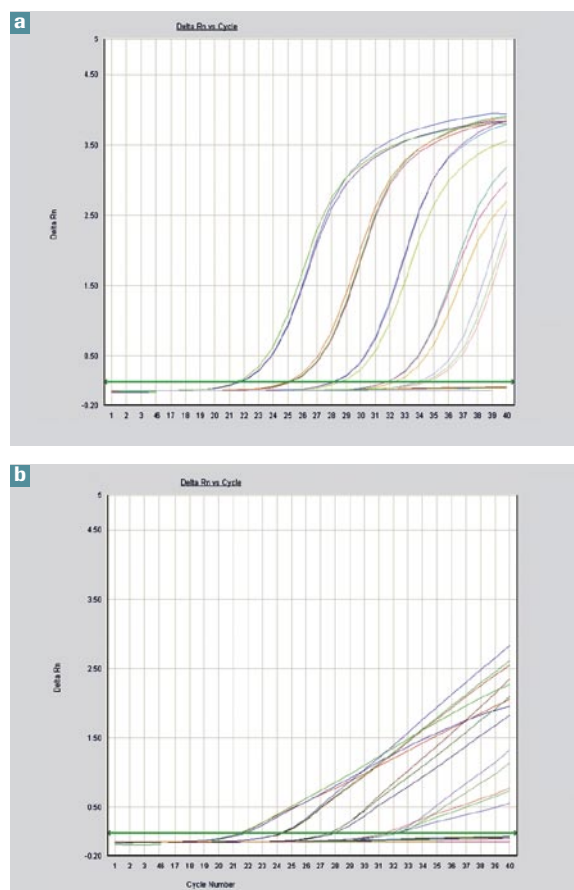
Combine accuracy and specificity with robust signal strength on ALL real-time PCR instruments

FastStart Universal SYBR Green Master (Rox)

This master is a ready-to-use, 2x-concentrated mix containing all the reagents (except primers and template) for running quantitative, real-time DNA detection assays, including qPCR and two-step qRT-PCR, in the SYBR Green I detection format. This formulation contains the universal reference dye, Rox. The performance of this master mix is ideal for all real-time instruments that require normalisation with a reference dye, including the Applied Biosystems 7900 HT Fast Real-Time PCR System or the Applied Biosystems 7500 Real-Time PCR System.

- **Improve PCR sensitivity and specificity.** Rely on this mix's FastStart Taq DNA Polymerase to minimize the formation of nonspecific amplification products through hot start PCR.
- **Amplify and detect a broad range of DNA or cDNA targets.** Amplify fragments up to 500 bp long, including those that are GC- or AT-rich.
- **Prevent false positives resulting from carryover contamination.** Add Uracil-DNA Glycosylase to degrade amplification products from previous PCR reactions produced with this dUTP-containing mix.

Product	Cat. No.	Pack Size
FastStart Universal SYBR Green Master (Rox)	04 913 850 001	5 ml (4 x 1.25 ml, for 500 reactions of 20 µl final volume)
	04 913 914 001	50 ml (10 x 5 ml, for 5,000 reactions of 20 µl final volume)



▲ **Figure 18: Quantification of the human globin gene** with the FastStart Universal SYBR Green Master (Rox) (a) and a master mix from another supplier (b) on an Applied Biosystems 7500 Real-Time PCR System.

The FastStart Universal SYBR Green Master (Rox) shows its higher sensitivity and more specific performance.

FastStart TaqMan® Probe Master

This master mix is a ready-to-use, 2x-concentrated mix and contains all the reagents (except primers, probe, and template) needed for running quantitative, real-time DNA-detection assays, including qPCR and two-step qRT-PCR, in the hydrolysis probe detection format.

- **Increase qPCR sensitivity and specificity.** Produce lower cycle threshold (Ct) values with the hot-start enzyme FastStart Taq DNA Polymerase and highest purity nucleotides included in the master mix.
- **Use it with any probe-based assay.** Achieve sensitive, specific results with Universal ProbeLibrary or any other hydrolysis probes.
- **Amplify and detect a broad range of DNA or cDNA targets.** Amplify fragments up to 500 bp long, including those that are GC- or AT-rich.

Product	Cat. No.	Pack Size
FastStart Taqman® Probe Master	04 673 409 001	2.5 ml (2 x 1.25 ml, for 100 reactions of 50 µl final volume)
	04 673 417 001	12.5 ml (10 x 1.25 ml, for 500 reactions of 50 µl final volume)
	04 673 433 001	50 ml (10 x 5 ml, for 2,000 reactions of 50 µl final volume)

FastStart SYBR Green Master

This master mix is ready-to-use, 2x-concentrated mix and contains all the reagents (except primers and template) needed for running quantitative, real-time DNA detection assays, including qPCR and two-step qRT-PCR, in the SYBR Green I detection format.

Product	Cat. No.	Pack Size
FastStart SYBR Green Master	04 673 484 001	5 ml (4 x 1.25 ml, for 200 reactions of 50 µl final volume)
	04 673 492 001	50 ml (10 x 5 ml, for 2,000 x 50 µl reactions)

Gene Expression Microarrays

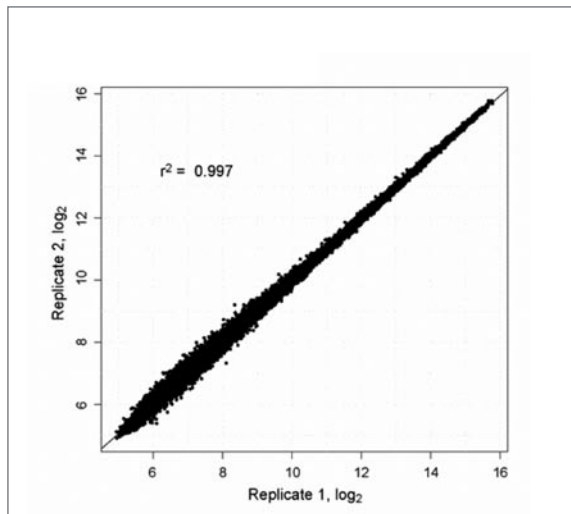
Identify genes of interest

NimbleGen Gene Expression Arrays

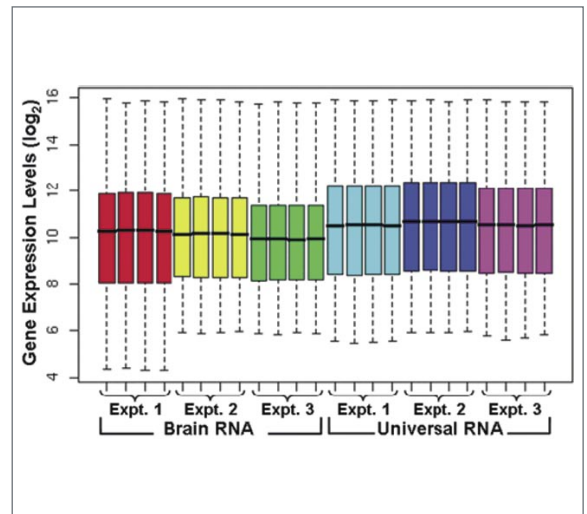
High-density arrays for accurate, sensitive, and reproducible gene expression profiling

As a first step in your gene expression analysis, identify genes of interest with Roche's line of gene expression analysis arrays. Rely on a powerful combination of high-density arrays, long-oligo probes, and flexible design capability providing superb results for advanced gene expression analysis. Choose rigorously tested technology from a world-renowned supplier, and achieve accurate, sensitive, and specific interrogation of genome-wide expression for any sequenced and annotated genome.

- **Benefit from ultra-high density.** Evaluate every gene with multiple, unique probes (6 to 12 probes per gene).
- **Maximize value.** Reduce cost-per-sample, and rely on exact manufacturing standards that ensure very high data reproducibility.
- **Enhance accuracy.** Rely on long oligos (60-mers) to provide high level signal-to-noise ratios and increased sensitivity, specificity, and reproducibility.



▲ **Figure 19: Accuracy of Gene Expression Levels from NimbleGen Microarrays.** The ratio of brain to universal expression values (\log_2) from NimbleGen Human Gene Expression 12 x 135 K microarrays were compared to TaqMan[®] data obtained from the MAQC report.¹ Results show a high concordance ($r = 0.92$) in gene expression levels between NimbleGen microarray and TaqMan[®] data.



▲ **Figure 20: Sensitivity of Gene Expression Levels from NimbleGen Microarrays.** Human brain and universal RNA¹ were analyzed on NimbleGen Human Gene Expression 12 x 135 K microarrays (4 technical replicates per sample). High sensitivity is demonstrated by the wide dynamic range (\log_2 of 4–16) of gene expression values on each array.
¹Nat. Biotechnol. 2006; 24(9): 1151–1161)

NimbleGen offers custom service for your microarray expression analysis needs. When the nine species specific expression arrays we offer do not meet your needs, rely on our flexible array scientists to design, or even run, microarrays for any sequenced and annotated genome. Our microarrays and genome services complement our existing LightCycler® 480 Real-Time PCR System and MagNA Pure automated nucleic acid purification solutions. The growing list of scientific publications (www.nimblegen.com) proves that Roche NimbleGen is the gene expression platform of choice. To order, or for more information, visit us at www.nimblegen.com/sales.



▲ Figure 21: NimbleGen Chips

NimbleGen Gene Expression 12x135K Microarrays			Part Number: 05224691001		
Eukaryotic Gene Expression 12x135K Catalog Designs					
Catalog Number	Organism	Sequence Source	Target Genes	Probes per Target Gene	Replicate Probe Sets per Array
A6484-00-01	Human	NCBI Build 36 (hg18)	44,049	3	1
A6485-00-01	Mouse	NCBI Build 37 (mm8)	42,576	3	1
A6486-00-01	Rat	Ensembl RGSC 3.4	26,419	5	1
A7239-00-01	<i>A. thaliana</i>	TAIR 6.0	30,380	4	1
A7237-00-01	<i>C. elegans</i>	Wormbase WS160	23,945	5	1
A7238-00-01	<i>D. melanogaster</i>	Flybase DM4.3	15,595	8	1
A7236-00-01	<i>S. cerevisiae</i>	NCBI Build October 2003	5,735	11	2
A7235-00-01	<i>S. pombe</i>	NCBI Build June 2005	4,976	12	2

Resources

The information provider for the life sciences

For more information about Roche Applied Science's complete line of tools for the gene expression analysis workflow, please refer to our special interest site at:

www.gene-expression.roche.com

Roche Applied Science is proud of its international network of dedicated application specialists and sales representatives. Please do not hesitate to contact us via telephone or the internet (www.roche-applied-science.com).

Our service specialists will be happy to assist you in streamlining your workflow, finding the best possible product combinations for your gene expression analysis needs.

Roche Prime Supply

To ensure immediate availability of all Roche Applied Science molecular biology reagents, contact your local representative about the Prime Supply Program. This procurement system uses an on-site user-friendly computerized interface to provide an uninterrupted supply of high-quality Roche biochemicals and research products.

For more information please visit:

www.roche-prime-supply.com



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Published by

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

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