microRNA Sample and Assay Technologies

miRNA purification, quantification,
and functional analysis

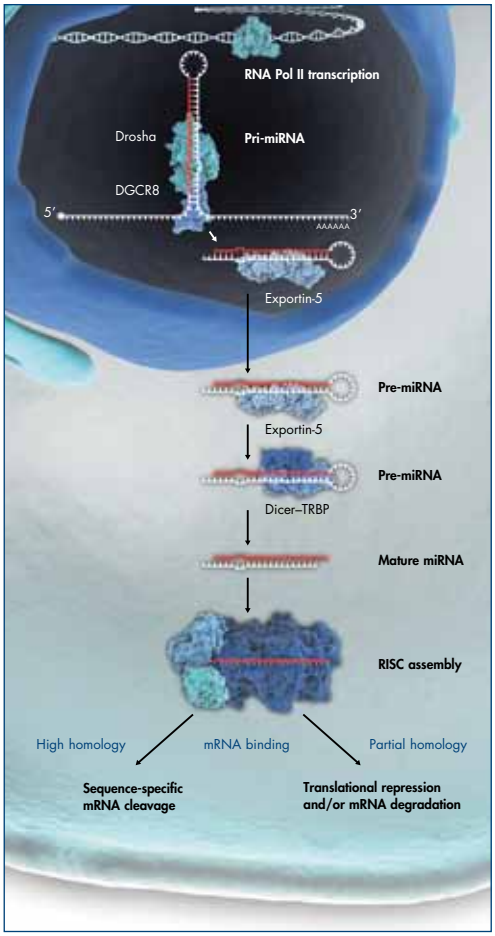
QIAGEN®

Sample & Assay Technologies

miRNA research from biogenesis to miRNome profiling

microRNAs (miRNAs) are naturally occurring, ~22 nucleotide, noncoding RNAs that mediate post-transcriptional gene regulation (Figure 1). miRNAs play an important role in many biological processes, including differentiation and development, cell signaling, and response to infection. Overwhelming evidence indicates that dysregulation of miRNA expression is a cause or indicator of several disease processes, including many cancers. The discovery that circulating miRNAs are detectable in serum and plasma, and that their expression varies as a result of disease, presents great potential for circulating miRNA expression signatures to be used as biomarkers in disease diagnosis and prevention.

Success of miRNA research relies on access to high-performance tools and technologies. QIAGEN is the one-stop provider for all your miRNA purification, quantification, and functional characterization needs. Visit QIAGEN's GeneGlobe® Web portal (www.qiagen.com/GeneGlobe) to order miRNA-specific products and to find a wealth of valuable gene information, pathway information, and bioinformatics tools.



QIAGEN provides solutions for:

- **miRNA purification**
  Purify high-quality total RNA, including miRNA, using miRNeasy Kits and PAXgene® Kits.
- **miRNA expression profiling**
  Rapidly profile the whole miRNome or pathway-focused miRNA panels using miScript® miRNA PCR Arrays.
- **miRNA biogenesis and regulation studies**
  Simultaneously quantify mature miRNAs (with miScript Primer Assays), precursor miRNAs (with miScript Precursor Assays), and mRNA targets (with QuantiTect® Primer Assays) using the miScript PCR System.
- **miRNA functional analysis**
  Determine the function of miRNAs using miScript miRNA Mimics, Inhibitors, and Target Protectors.

Figure 1. The canonical pathway of miRNA biogenesis. miRNAs are transcribed as long, primary miRNAs (pri-miRNAs) that are processed into precursor miRNA stem loops (pre-miRNAs) by Drosha–DGCR8 in the nucleus. The resulting pre-miRNAs are exported by Exportin-5-Ran-GTP into the cytosol, and are further processed into ~22 nucleotide, mature miRNAs by Dicer–TRBP. These mature miRNAs are assembled into Ago2-containing RNA-induced silencing complexes (RISC). RISC-associated miRNA binds to the 3'-untranslated region of a target mRNA resulting in post-transcriptional gene regulation. The extent to which an miRNA can base pair with its target mRNA determines whether regulation takes place by mRNA cleavage (perfect/near-perfect base pairing to the target) or translational repression/mRNA destabilization (imperfect base pairing to the target).

miScript PCR System — miRNA quantification redefined

The next-generation miScript PCR System allows sensitive and specific quantification and profiling of miRNA expression using SYBR® Green-based real-time PCR. miScript miRNA PCR Arrays enable rapid profiling of the complete miRNome or pathway-focused panels of mature miRNAs for a variety of species. Additionally, individual assays for mature miRNAs, precursor miRNAs, and other small noncoding RNAs (ncRNA) can be ordered at the GeneGlobe Web portal (www.qiagen.com/GeneGlobe).

The miScript PCR System offers:

- Sensitive profiling of mature miRNA expression using miRNome and pathway-focused PCR arrays
- Unmatched flexibility to quantify mature miRNA, precursor miRNA, ncRNA, and mRNA from a single cDNA sample
- Free, easy-to-use data analysis tools for miScript miRNA PCR Arrays

One system for all your miRNA quantification needs

The robust miScript PCR System covers all the steps of miRNA quantification from conversion of RNA into cDNA to real-time PCR detection of miRNAs and straightforward data analysis. The components of the miScript PCR System are detailed below.

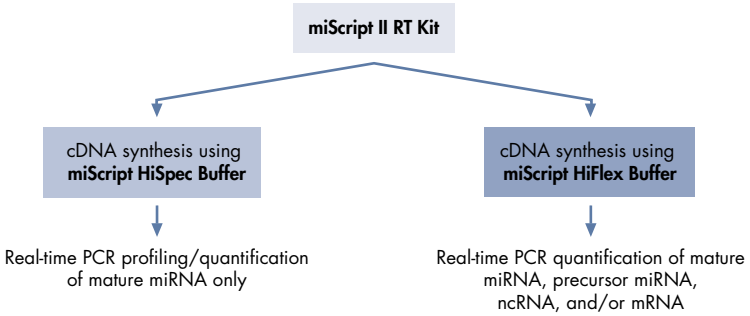


Figure 2. miScript II RT Kit dual-buffer system. Use miScript HiSpec Buffer for cDNA synthesis followed by mature miRNA quantification using miScript miRNA PCR Arrays or miScript Primer Assays. Use miScript HiFlex Buffer for cDNA synthesis followed by quantification of mature miRNA, precursor miRNA, other noncoding RNA (ncRNA), and/or mRNA from the same cDNA (using appropriate primer assays).

miScript II RT Kit

The miScript II RT Kit is used to perform a one-step, single-tube reverse transcription reaction. The dual-buffer system has been developed to facilitate both rapid profiling of mature miRNA expression and flexible quantification of all RNA species using real-time PCR (Figure 2).

miScript HiSpec Buffer facilitates the selective conversion of mature miRNAs into cDNA. This enables rapid profiling of mature miRNA with virtually no background signal. miScript HiFlex Buffer promotes conversion of all RNA species (mature miRNA, precursor miRNA, noncoding RNA, and mRNA) into cDNA. This enables unrivaled flexibility to study miRNA biogenesis and genomewide miRNA and mRNA regulation in a single cDNA sample.

Find out more about the miScript II RT Kit at www.qiagen.com/miScriptRT.

miScript SYBR Green PCR Kit

The miScript SYBR Green PCR Kit includes QuantiTect SYBR Green PCR Master Mix and the miScript Universal Primer, a reverse PCR primer that allows detection of mature miRNAs in combination with miScript Primer Assays (mature miRNA-specific forward primers). This kit is optimized to provide high-quality detection of mature miRNA using real-time PCR.

Find out more about the miScript SYBR Green PCR Kit at www.qiagen.com/miScriptPCR.

miScript Primer Assays and miScript Precursor Assays

miScript Primer Assays are mature-miRNA–specific forward primers. Used in combination with the miScript Universal Primer (reverse primer), miScript Primer Assays enable specific detection of mature miRNA. miScript Precursor Assays are precursor-miRNA–specific forward and reverse primer pairs that enable specific quantification of precursor miRNA stem loops. All assays are designed using the most up-to-date sequence information from miRBase and are available at the GeneGlobe Web portal (www.qiagen.com/GeneGlobe).

Find out more about miScript Assays at www.qiagen.com/miScriptAssays and www.qiagen.com/miScriptPrecursor.

miScript miRNA PCR Arrays

miScript miRNA PCR Arrays.

Cutting-edge miScript miRNA PCR Arrays consist of mature miRNA-specific forward primers (miScript Primer Assays) arrayed in miRNome panels and biologically relevant, pathway-focused panels. Used in combination with the miScript SYBR Green PCR Kit, miScript miRNA PCR Arrays enable rapid, specific, and reproducible profiling of mature miRNA expression. Complete confidence in your results is assured, as each assay in a miScript miRNA PCR Array has been extensively bench validated to ensure sensitive and specific detection of mature miRNA.

Find out more about miScript miRNA PCR Arrays at www.qiagen.com/miScriptArrays.

miScript PCR Controls

miScript PCR Controls are primers that enable normalization of real-time PCR results in miRNA quantification studies using the miScript PCR System. miScript PCR Controls are available for 5 snoRNAs and one snRNA. These controls can be used for normalization in human, mouse, rat, dog, and rhesus macaque studies.

For the most up-to-date list of species and more information, visit www.qiagen.com/miScriptControls.

miScript miRNA PCR Array Data Analysis Tool

Free, easy-to-use data analysis software is available online for miScript miRNA PCR Arrays. Each miScript miRNA PCR Array comes with a content-specific data analysis tool. Once raw C_T values are uploaded, the analysis tool automatically interprets the PCR array controls, performs relative quantification using the $\Delta\Delta C_T$ method, and presents the results in a variety of visual formats, allowing you to rapidly interpret your data.

Find out more about data analysis at www.qiagen.com/miScriptAnalysis.

Cutting-edge miRNA expression profiling tools

miRNA expression profiling using miScript miRNA PCR Arrays

miScript miRNA PCR Arrays are mature-miRNA–specific, forward primers (miScript Primer Assays) that have been arrayed in miRNome and biologically relevant, pathway-focused panels (Table 1). These PCR arrays are provided in ready-to-use, 384-well plate, 96-well plate, and 100-well Rotor-Disc® formats (Table 2 and Figure 3). miScript miRNA PCR Arrays are available for various species, provide guaranteed high performance, and are fully customizable. Each array contains controls that allow monitoring of the complete experiment from sample prep to data analysis. These controls include data normalization controls, reverse transcription controls, and PCR controls. Every assay in a miScript miRNA PCR Array has been bench validated to ensure sensitive and specific detection of mature miRNA by real-time PCR.

Table 1. Available miScript miRNA PCR Arrays

Array	Species
Complete miRNome	Human, mouse, rat, dog, rhesus macaque
miFinder	Human, mouse, rat, dog, rhesus macaque
Brain Cancer	Human, mouse, rat
Breast Cancer	Human, mouse, rat
Cancer PathwayFinder	Human, mouse, rat
Cell Differentiation & Development	Human, mouse, rat
Immunopathology	Human, mouse, rat
Inflammatory Response & Autoimmunity	Human, mouse, rat
Neurological Development & Disease	Human, mouse, rat
Ovarian Cancer	Human, mouse, rat
Serum & Plasma	Human, mouse, rat
Custom Array	Human, mouse, rat, dog, rhesus macaque, and other species

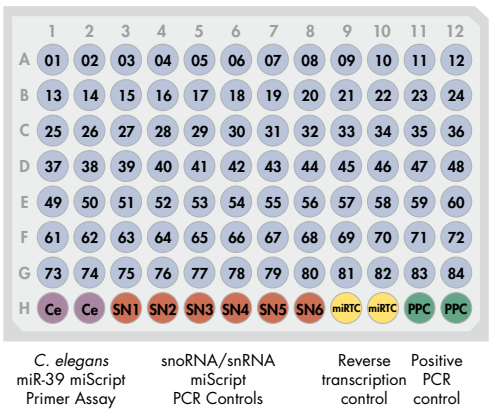


Figure 3. miScript miRNA PCR Array layout. miScript miRNA PCR Arrays provide miScript Primer Assays for 84 miRNAs, as well as controls for data normalization, reverse transcription, and PCR. Data normalization controls include 6 miScript PCR Controls (see page 10) and a primer assay for *C. elegans* miR-39. The cel-miR-39 assay detects the Syn-cel-miR-39 miScript miRNA Mimic, which can be used as a spike-in control. Rotor-Disc and 384-well formats are also available.

Table 2. Real-time cyclers compatible with miScript miRNA PCR Arrays

Manufacturer	Cycler	Format
QIAGEN	Rotor-Gene® Q, Rotor-Gene 6000	R
Applied Biosystems	ABI™ 5700, 7000, 7300, 7500 Standard, 7900HT Standard (96-well block), 7700, ViiA™ 7 (96-well block)	A
	ABI 7500 Fast, 7900HT Fast (96-well block), ViiA 7 Fast (96-well block), StepOnePlus™	C
	ABI 7900HT, ViiA 7 (384-well block)	E
Bio-Rad	iCycler®, iQ™ 5, MyiQ™, MyiQ2, Chromo4™	A
	CFX96™, DNA Engine Opticon®, DNA Engine Opticon 2	D
	CFX384™	E
Agilent (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

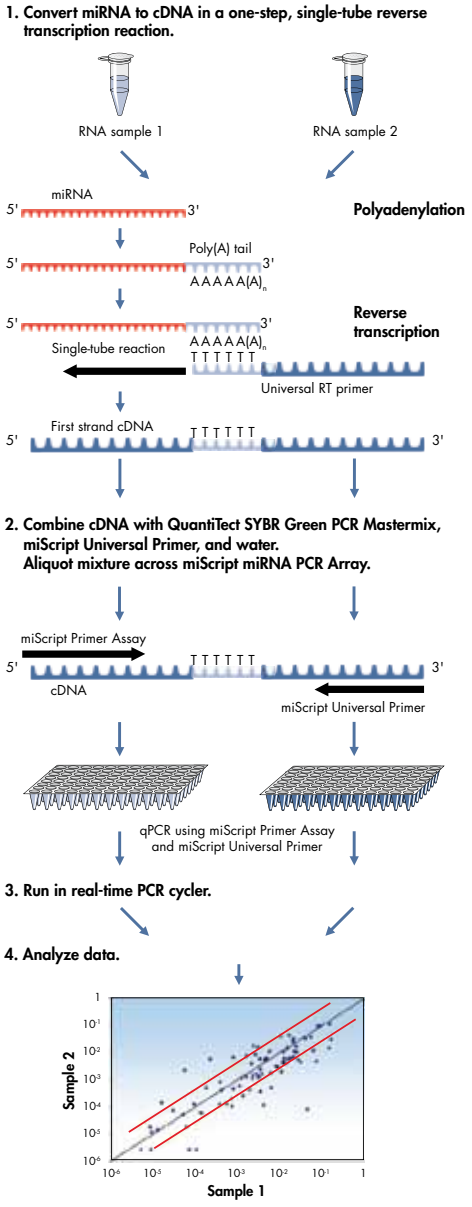


Figure 4. miScript miRNA PCR Array workflow.

Straightforward, rapid workflow

miRNA expression profiling with miScript miRNA PCR Arrays is simple and robust (Figure 4). First, prepare cDNA with the miScript II RT Kit using miScript HiSpec Buffer. Second, add a premix of cDNA, miScript Universal Primer, QuantiTect SYBR Green PCR Master Mix, and RNase-free water to a miScript miRNA PCR Array. Third, run the reaction in a real-time PCR cycler. Finally, analyze the data using the miScript miRNA PCR Array Data Analysis Tool. Built with the end-user in mind, miScript miRNA PCR Arrays are at the forefront of real-time PCR-based, mature miRNA profiling tools.

Discover miRNA biomarkers in serum or plasma samples

The range of pathway-focused miScript miRNA PCR Arrays is continually expanding to enable new discoveries about the roles of miRNAs in biological processes. The content of miScript miRNA PCR Arrays is selected using our proprietary methodology, which ensures that the arrays are up-to-date and biologically relevant. One of the most exciting areas of current miRNA research involves the assessment of miRNAs present in serum or plasma samples. The presence of relatively stable, extracellular miRNAs in serum and plasma has generated great interest in the potential use of changes in these miRNA levels as noninvasive biomarkers for a variety of diseases. As a result, the Serum & Plasma miScript miRNA PCR Array has been developed to enable rapid profiling of the 84 most relevant, disease-associated miRNAs in serum and plasma (Figure 5).

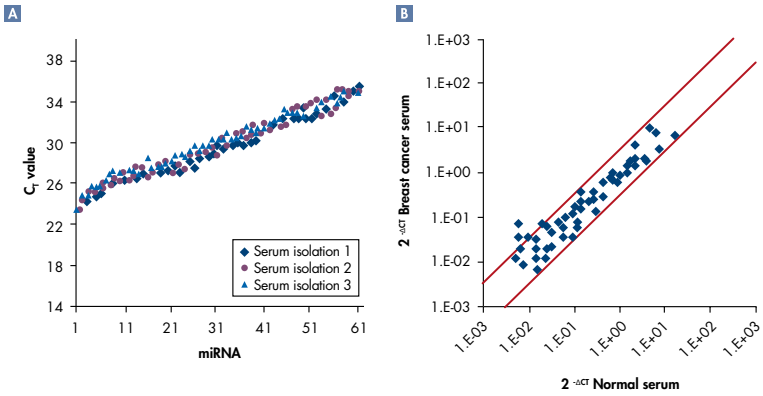


Figure 5. miRNA biomarker discovery in serum samples. Total RNA was isolated from normal (n=10) and breast cancer (n=3) serum samples. cDNA was prepared with the miScript II RT Kit using the provided miScript HiSpec Buffer. The Human Serum & Plasma miScript miRNA PCR Array, in combination with the miScript SYBR Green PCR Kit, was used to profile mature miRNA expression by real-time PCR. **A** C_T values from 3 normal serum samples demonstrate high reproducibility. **B** A scatter plot of $2^{-\Delta CT}$ values demonstrates that significant differences exist between mature miRNA expression levels in the normal serum and breast cancer serum pools (±3-fold difference in expression indicated by red lines).

Unlock miRNA expression data archived in FFPE samples

Formalin-fixed paraffin-embedded (FFPE) tissue samples are a valuable source of sample material for retrospective miRNA expression profiling. QIAGEN provides a complete solution for FFPE sample analysis. The miRNeasy FFPE Kit enables purification of total RNA, and the yields and performance are superior to alternative methods of miRNA purification, such as using phenol-chloroform extraction. miScript miRNA PCR Arrays can then unlock the valuable miRNA data found in the FFPE samples (Figure 6).

Achieve maximum miRNA expression profiling in your model system

In addition to pathway-focused arrays, up-to-date miRNome miScript miRNA PCR Arrays are available for a variety of species including human, mouse, rat, dog, and rhesus macaque. With less than 1 µg total RNA, the entire human miRNome can be rapidly profiled (Figure 7).

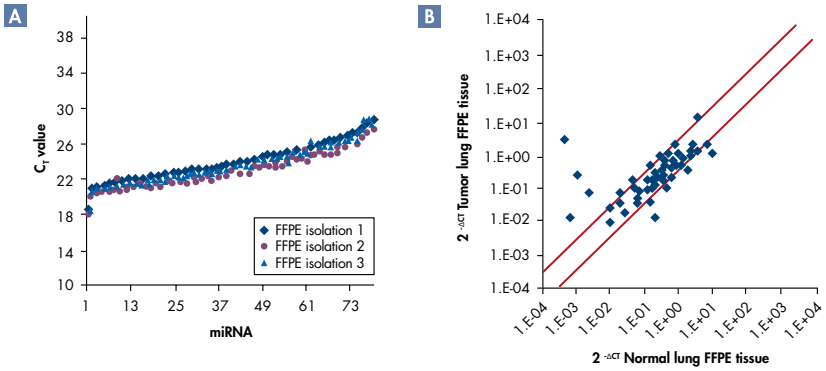


Figure 6. miRNA expression profiling in FFPE samples. Total RNA was isolated from thin (5 µm) normal and tumor lung FFPE tissue sections using the miRNeasy FFPE Kit. cDNA was prepared with the miScript II RT Kit using the provided miScript HiSpec Buffer. The Human miFinder miScript miRNA PCR Array, in combination with the miScript SYBR Green PCR Kit, was used to profile mature miRNA expression by real-time PCR. A C_T values from 3 tumor lung FFPE samples demonstrate high reproducibility. B A scatter plot of $2^{-\Delta CT}$ values comparing normal and tumor samples demonstrates that significant differences exist between mature miRNA expression levels in the 2 tissue types (±3-fold difference in expression is indicated by red lines).

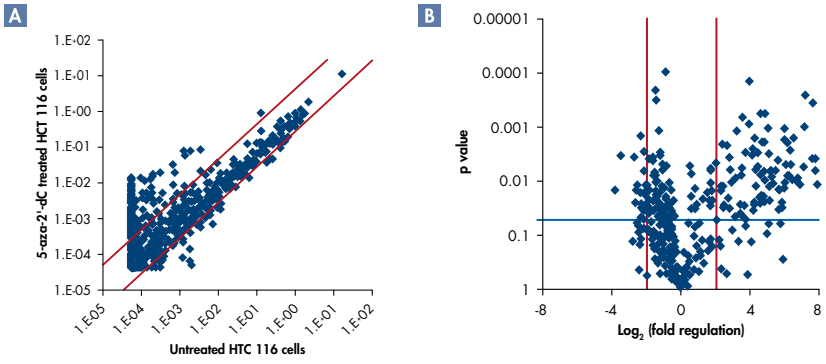


Figure 7. miRNA expression profiling in a model system. Using the miRNeasy Mini Kit, total RNA was isolated from HCT 116 colorectal cancer cells that had been treated ± 5-aza-2'-deoxycytidine (5-aza-2'-dC) demethylation reagent. This reagent irreversibly inhibits DNA methyltransferase driven methylation reactions by incorporating into DNA and covalently binding to the active site of the DNA methyltransferase. cDNA was prepared with the miScript II RT Kit using the provided miScript HiSpec Buffer. The Human miRNome miScript miRNA PCR Array, in combination with the miScript SYBR Green PCR Kit, was used to profile mature miRNA expression by real-time PCR. A A scatter plot of $2^{-\Delta CT}$ values demonstrates that significant differences exist in the mature miRNA expression levels of the 2 samples. B A volcano plot shows regulated miRNAs (±4-fold indicated by red lines). In 5-aza-2'-dC treated HCT 116 cells, 104 miRNAs were strongly upregulated and 30 were strongly downregulated. When a p value of 0.05 is applied (indicated by blue line), the upregulation of 89 of the 104 miRNAs is significant, and the downregulation of 21 of the 30 miRNAs is significant.

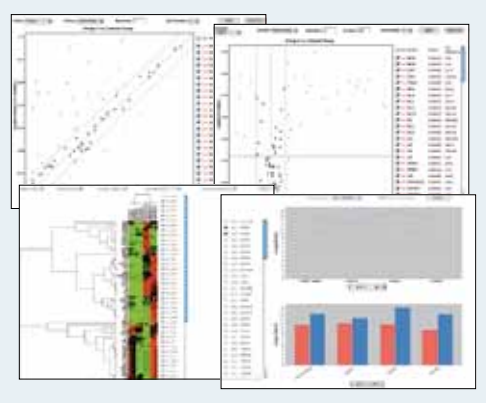


Figure 8. Data analysis made easy with Web-based software.

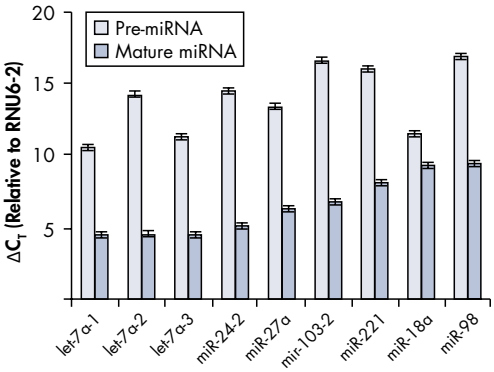


Figure 9. Detection of mature and precursor miRNAs. Total RNA was purified from HeLa S3 cells using the miRNeasy Mini Kit. cDNA was prepared using the miScript II RT Kit with the provided miScript HiFlex Buffer. In real-time PCR, miScript Primer Assays were used for mature miRNA quantification, miScript Precursor Assays were used for precursor miRNA quantification, and the miScript PCR Control for RNU6-2 was used for normalization. Relative levels of mature and precursor miRNA vary for different miRNAs, and different precursors, expressed at different genomic loci, can generate the same mature miRNA.

Simplify your real-time PCR data analysis

A free data analysis tool for each miScript miRNA PCR Array is available online. The data analysis tool, provided in Web-based and Excel® formats, automatically performs quantification using the $\Delta\Delta C_T$ method of relative quantification (Figure 8).

miScript miRNA PCR Array Data Analysis Tools automatically:

- Interpret control wells
- Calculate fold-changes using the $\Delta\Delta C_T$ method
- Present results in a variety of visual formats

Uncover the mechanisms of miRNA biogenesis

For experiments to study the relationship of precursor miRNA to mature miRNA, it is necessary to have the flexibility to detect both types of miRNA from the same cDNA sample. cDNA prepared with miScript HiFlex Buffer using the miScript II RT Kit provides this flexibility. In real-time PCR, mature miRNAs are detected using miScript Primer Assays, and precursor miRNAs are detected using miScript Precursor Assays (Figure 9). Individual miScript Primer Assays and miScript Precursor Assays are available online at the GeneGlobe Web portal. Relative levels of mature and precursor miRNA vary for different miRNAs. In addition, mature miRNAs may be expressed from different precursor miRNAs under different conditions or in different tissues (Figure 9).

Explore the miScript PCR System using the miScript PCR Starter Kit

For scientists new to the field of miRNA research, the miScript PCR Starter Kit provides all the components necessary to perform miRNA quantification experiments. The kit includes reagents for reverse transcription and real-time PCR, as well as a miScript Primer Assay for human miR-15a and the miScript PCR Control for RNU6-2. The kit allows establishment and monitoring of experimental setup. It is an ideal tool for expanding into the exciting field of miRNA research.

Sensitive, reproducible miRNA quantification

A robust miRNA detection system must be sensitive and have a broad dynamic detection range. In addition, primers should have efficiencies near 100%, as well as the ability to discriminate between miRNA isoforms. The quantification workflow should also enable easily reproducible experiments. The miScript PCR System demonstrates all these benefits, whether it is used for miRNA expression profiling or biogenesis studies. As little as 10 miRNA copies can be detected, whether cDNA is prepared using miScript HiSpec Buffer or miScript HiFlex Buffer (Figure 10). Only 10 pg total RNA is required to produce robust data with primer amplification efficiencies close to 100%. In addition to sensitivity, high primer specificity is also assured. The miScript PCR System can efficiently discriminate between closely related isoforms of miRNA family members, even when they differ by only a single base (Table 3). Even a first-time user of the miScript PCR System can generate technical and biological replicate results that are virtually indistinguishable (Figure 11).

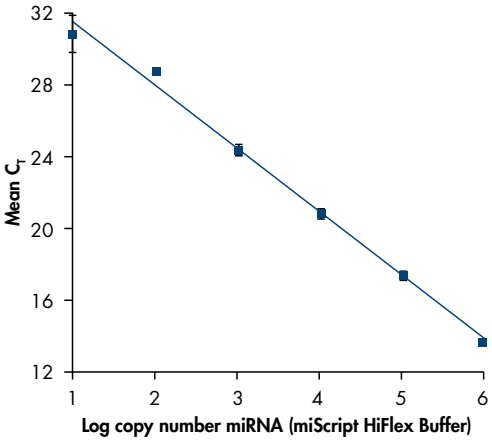


Figure 10. Accurate detection of as low as 10 miRNA copies. Synthetic miR-21 was used to generate cDNA using the miScript II RT Kit with miScript HiFlex Buffer (provided in the kit). A range of amounts from 10 copies to 10^6 copies of this cDNA was used in real-time PCR using the miScript PCR System. The resultant C_T values decreased linearly with increasing miRNA copy number, indicating sensitive detection across a wide range of template amounts. Similar results were achieved using miScript HiSpec Buffer (data not shown).

Table 3. Specificity of miScript Primer Assays

cDNA used	% Activity relative to perfect match primer assay as 100%								
	miScript Primer Assay used								
	Let-7b	Let-7c	miR-98	Let-7d	Let-7e	Let-7a	Let-7f	Let-7g	Let-7i
Let-7b	**100.0**	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Let-7c	0.5	**100.0**	0.0	0.0	2.4	0.1	0.0	0.0	0.0
miR-98	0.0	0.2	**100.0**	0.1	0.0	0.1	0.0	0.0	0.1
Let-7d	0.1	0.0	0.0	**100.0**	0.0	0.4	0.0	0.0	0.0
Let-7e	0.1	0.0	0.0	0.0	**100.0**	0.2	0.0	0.0	0.0
Let-7a	0.1	0.6	0.0	0.5	3.1	**100.0**	0.1	0.0	0.0
Let-7f	0.6	0.1	0.0	0.1	0.0	1.0	**100.0**	0.1	0.1
Let-7g	0.6	0.2	0.0	0.1	0.0	0.0	0.0	**100.0**	0.2
Let-7i	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1	**100.0**

Let-7 family members include isoforms that differ by as little as one base. Synthetic miRNAs of each Let-7 isoform (Let-7a–Let-7i, miR-98) were used to generate cDNA with the miScript II RT Kit using miScript HiFlex Buffer. The same experiment performed using miScript HiSpec Buffer gave similar results (data not shown). Each cDNA was used as template in real-time PCR reactions with a miScript Primer Assay for each isoform. The % relative detection was calculated using the differences between the C_T values achieved from the mismatching miScript Primer Assays and those from the perfectly matching miScript Primer Assays (% relative detection = $2^{-\Delta CT}$ x 100). The miScript PCR System efficiently discriminated between closely related isoform family members.

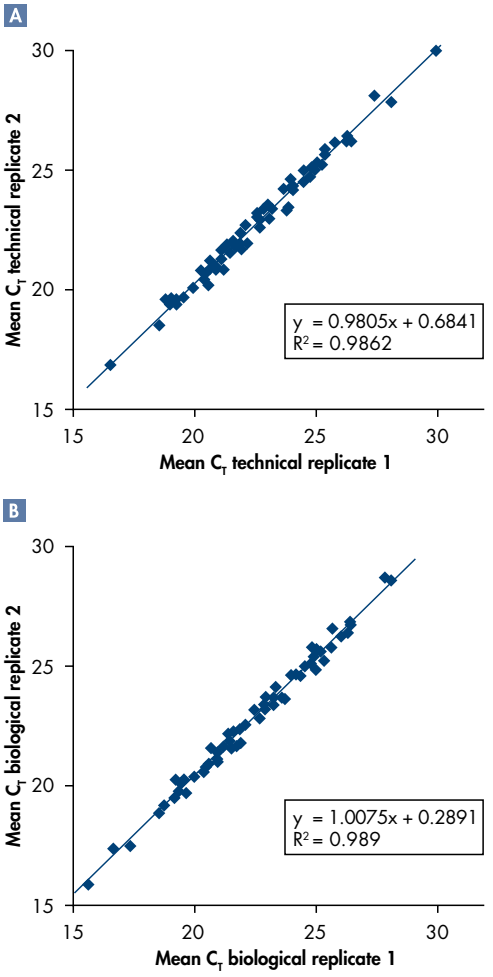


Figure 11. Reproducible results in technical and biological replicates. Total RNA was purified using the miRNeasy Mini Kit from A 2 identical HeLa S3 cell pellets with identical passage numbers and B 2 HeLa S3 cell pellets collected from different parental stocks at different times. cDNA was prepared using the miScript II RT Kit with the provided miScript HiSpec Buffer. The miFinder miScript miRNA PCR Array was used for miRNA profiling. Plotting C_T values of the replicates against each other demonstrated the high technical and biological reproducibility of the results. These experiments were performed by a first-time user of the miScript PCR System.

Let QIAGEN profile your miRNA

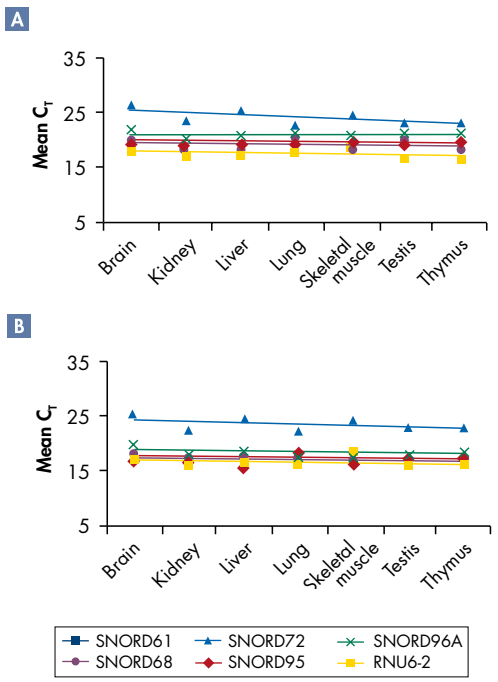
The RNA Isolation Service and miScript miRNA PCR Array Service comprise a team of highly trained, QIAGEN scientists with the expertise to purify nucleic acids from your biological samples and perform miRNA profiling.

QIAGEN miRNA Services provide the following sample-to-result solutions:

- Total RNA purification using QIAGEN kits (miRNeasy or PAXgene Kits)
- Reverse transcription using the miScript II RT Kit
- miRNome or pathway-focused expression profiling using miScript miRNA PCR Arrays
- Data analysis using the miScript miRNA PCR Array Data Analysis Tool

Normalization controls for miRNA quantification

For accurate and reproducible results in miRNA quantification by real-time PCR, it is necessary to normalize the amount of target miRNA by using a suitable endogenous reference RNA. This approach is known as relative quantification. Normalization corrects for factors that could otherwise lead to inaccurate quantification and also allows results from different experiments and samples to be compared directly. miScript PCR Controls enable normalization of human, mouse, rat, dog, and rhesus macaque real-time PCR miRNA expression data when using the miScript PCR System. For the most up-to-date list of species, visit www.qiagen.com/miScriptControls.



Advantages of miScript PCR Controls:

- All controls effective in human, mouse, rat, dog, and rhesus macaque
- High amplification efficiencies of close to 100%
- Constant expression levels in a variety of cells and tissues

Confidence in your results

miScript PCR Controls are primers designed to quantify a panel of 5 snoRNAs and one snRNA. These RNAs have relatively stable expression levels across tissues and cell types (Figure 12). miScript PCR Controls provide amplification efficiencies of close to 100%. cDNA prepared with either miScript HiSpec Buffer or miScript HiFlex Buffer can be used with miScript PCR Controls. miScript PCR Controls are included on all miScript miRNA PCR Arrays (Figure 3) and can also be ordered individually at the GeneGlobe web portal (www.qiagen.com/GeneGlobe).

Figure 12. Constant expression levels of targeted RNAs. Total RNA (200 ng) from various human tissues was converted into cDNA using the miScript II RT Kit with A miScript HiSpec Buffer or B miScript HiFlex Buffer (provided in the kit). Real-time PCR was performed using 1 ng cDNA with the miScript PCR System and each of the miScript PCR Controls. C_T values show that the expression level of each RNA was very similar in different tissue types. Similar results were achieved for mouse, rat, dog, and rhesus macaque tissues (data not shown).

Pinpoint miRNA roles in functional studies

miRNA functional studies enable you to identify the targets and roles of particular miRNAs, analyze the biological effects of misregulation of individual miRNAs, and confirm that specific genes are targets of particular miRNAs. Functional studies can be performed using miScript miRNA Mimics, miScript miRNA Inhibitors, and miScript Target Protectors (Figure 13). A variety of scales and formats can be ordered including tubes, 96-well plates, and 384-well plates.

Strategies to uncover miRNA function:

- Simulate endogenous miRNA regulation using miScript miRNA Mimics
- Suppress endogenous miRNAs using miScript miRNA Inhibitors
- Protect a single miRNA target gene using miScript Target Protectors

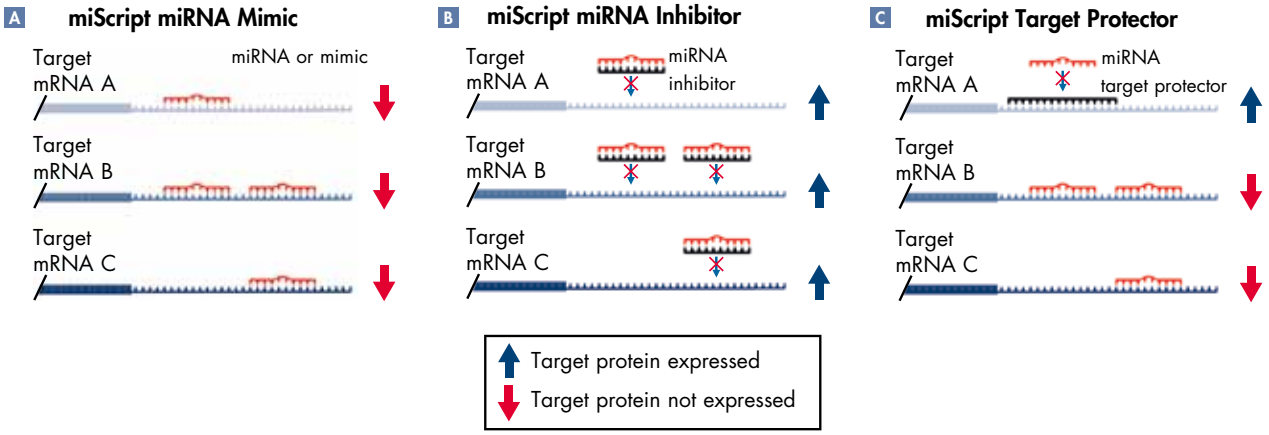


Figure 13. Mechanism of action of miScript miRNA Mimics, miScript miRNA Inhibitors, and miScript Target Protectors. A miRNAs or miScript miRNA Mimics bind to their target mRNAs, causing downregulation of expression of all the targets. B miScript miRNA Inhibitors bind to the miRNA of interest, resulting in the expression of all gene targets of the miRNA. C In contrast, miScript Target Protectors bind to a specific miRNA binding site, resulting in expression of that miRNA target only, while all other targets remain downregulated.

Replicate endogenous miRNA function using miScript miRNA Mimics

miScript miRNA Mimics are synthetic, double-stranded RNAs that mimic naturally occurring miRNAs after transfection into cells (Figure 13). miScript miRNA Mimics cause a decrease in gene expression similar to that observed with endogenous miRNAs (Figure 14). Gene expression is efficiently inhibited for up to 72 hours after transfection (data not shown).

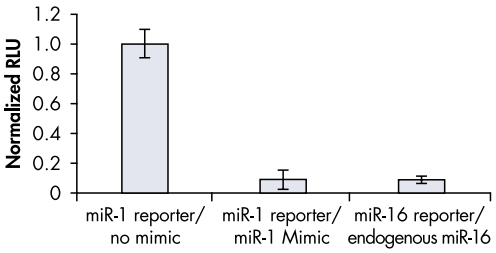


Figure 14. Comparable downregulation by endogenous miRNA and miScript miRNA Mimic. miR-1 is not endogenously expressed in HeLa S3 cells. miR-16 is endogenously expressed at high levels in HeLa S3 cells. Using HiPerFect Transfection Reagent, HeLa S3 cells (6 x 10^4 cells/well) were transfected with a luciferase reporter construct with a binding site for miR-1 in the 3' UTR; cotransfected with a luciferase reporter construct with a binding site for miR-1 in the 3' UTR and with an miR-1 Mimic; or transfected with a luciferase reporter construct with a binding site for miR-16 in the 3' UTR. Twenty-four hours later, luciferase activity was measured. miR-1 Mimic resulted in a similar decrease in luciferase level as endogenous miR-16.

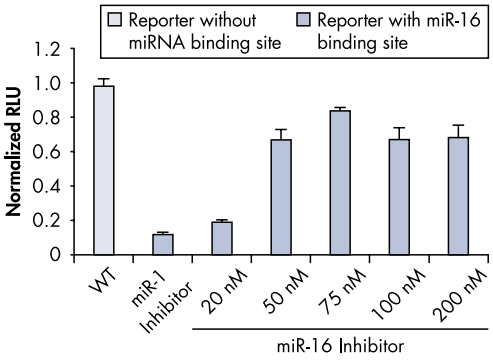


Figure 15. miScript miRNA Inhibitor counteracts miRNA-induced silencing. HeLa S3 cells express miR-16 at high levels and do not express miR-1. In a cotransfection experiment using HiPerFect Transfection Reagent, HeLa S3 cells (6 x 10^4 cells/well) were cotransfected with a luciferase reporter construct with an miR-16 binding site in the 3' UTR together with miR-16 Inhibitor. miR-16 Inhibitor was used at varying concentrations in the experiment to evaluate the optimal inhibitor concentration required to see the inhibitory effect. Alternatively, cells were transfected with miR-1 Inhibitor alone as a control. A luciferase construct without an miRNA binding site (**WT**) was transfected as a positive control. An increase in luciferase expression following transfection of the miR-16 Inhibitor indicated that it prevented endogenous miR-16 from downregulating the reporter gene.

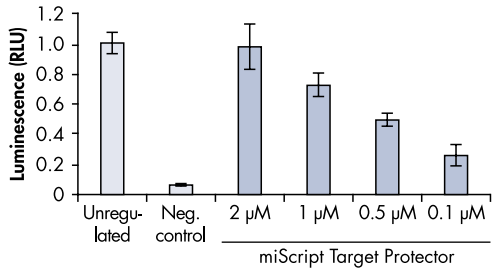


Figure 16. Reliable miRNA inhibition. miR-16 is endogenously expressed in MCF-7 cells. A luciferase reporter construct with a binding site for miR-16 was cotransfected with various amounts of a miScript Target Protector for the same binding site. All amounts resulted in significant increase in luciferase expression caused by inhibition of miRNA downregulation. **Neg. control**: Luciferase construct cotransfected with Negative Control miScript Target Protector. **Unregulated**: Luciferase construct without the miR-16 binding site. Attractene Transfection Reagent was used for cotransfections.

Block endogenous miRNA using miScript miRNA Inhibitors

miScript miRNA Inhibitors are synthetic, single-stranded, chemically modified RNAs that specifically inhibit miRNA function after transfection into cells (Figure 13, page 11). miScript miRNA Inhibitors have been designed and modified to ensure efficient inhibition of endogenous miRNA function (Figure 15). Time-course experiments indicate that miScript miRNA Inhibitors are effective for at least 96 hours following transfection (data not shown).

Selectively protect an miRNA target using a miScript Target Protector

miScript Target Protectors are single-stranded, modified RNAs that specifically interfere with the interaction of an miRNA with a single target, while leaving the regulation of other targets of the same miRNA unaffected (Figure 13, page 11). After transfection, a miScript Target Protector binds to its specific miRNA-binding site, blocking miRNA access to the site and preventing gene downregulation by a specific miRNA (Figures 16 and 17). miScript Target Protector transfection is followed by phenotype or gene expression analysis. Increased target-gene expression, a change in signaling patterns, or an altered phenotype, can all provide evidence that the miRNA and target under study are involved in the pathway or phenotype of interest.

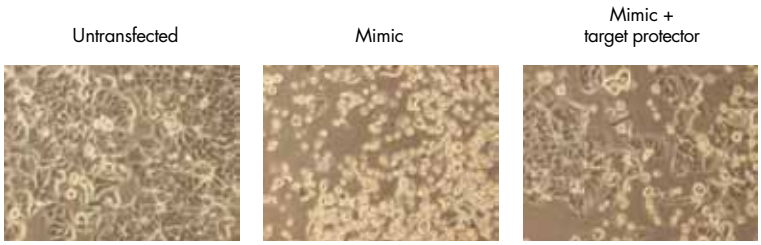


Figure 17. Phenotype changes after miScript Target Protector transfection. miR-9 causes downregulation of a gene that is important for cell viability. MCF-7 cells were transfected with 10 nM miR-9 miScript miRNA Mimic or cotransfected with 10 nM miR-9 miScript miRNA Mimic plus 1 µM miScript Target Protector for the miR-9 binding site of the cell viability gene. After 72 hours, cell viability was examined by light microscopy. Transfections were performed using HiPerFect Transfection Reagent. Transfection of miR-9 miScript miRNA Mimic resulted in high levels of cell death. However, cotransfection of miR-9 miScript miRNA Mimic and a miScript Target Protector designed for the miR-9 binding site of the gene overcame the cell death phenotype. The miScript Target Protector inhibited miR-9 downregulation allowing the cell viability gene to be expressed.

High-quality miRNA purification

Purification of high-quality, total RNA, including miRNA, is a prerequisite for successful miRNA quantification experiments, as poor quality RNA can hinder enzymatic reactions and inefficient purification of small RNA may lead to inaccurate results. miRNeasy and PAXgene miRNA Kits for miRNA purification ensure the highest quality RNA for your experiments (Table 4). miRNA yields are superior to alternative methods of miRNA purification, such as phenol-based extraction and precipitation (Figures 18 and 19). miRNA purification can be automated, if required, on the QIAcube® or QIAsymphony® instruments (for more information, visit www.qiagen.com/automation).

miRNeasy and PAXgene Kits for miRNA purification provide:

- High-purity RNA
- RNA suitable for all downstream applications
- Purification from a variety of sample types

Table 4. Kits for miRNA purification

Kit	Sample type
miRNeasy Mini Kit	Animal/human tissues and cells
miRNeasy 96 Kit	Animal/human tissues and cells
miRNeasy Micro Kit	Small amounts of animal/human tissues and cells
miRNeasy Serum/ Plasma Kit	Serum, plasma, and other body fluids
miRNeasy FFPE Kit	Formalin-fixed, paraffin-embedded (FFPE) tissue samples
PAXgene Blood miRNA Kit*	Human whole blood (stabilized in PAXgene Blood RNA Tubes)
PAXgene Tissue miRNA Kit	Animal/human tissues (fixed and stabilized in PAXgene Tissue Containers)

* For automated purification from human whole blood on the QIAsymphony, we recommend the QIAsymphony PAXgene Blood RNA Kit.

Purification of total RNA or enrichment of miRNA

miRNeasy and PAXgene miRNA Kits enable purification of total RNA, which includes RNA from approximately 18 nucleotides (nt) upwards. Alternatively, for all sample types, except samples containing severely degraded RNA (e.g., FFPE samples) and samples containing low amounts of large RNAs (e.g., serum/plasma samples), an miRNA-enriched fraction (<~200 nt) and a total RNA fraction (>~200 nt) can be purified separately using miRNeasy Kits. RNA purified using miRNeasy and PAXgene miRNA Kits is suitable for use in downstream applications, such as northern blot analysis, microarray analysis, and quantitative, real-time RT-PCR. Enrichment of small RNAs may reduce background in certain applications (e.g., some microarray systems), but is not required for analysis using miScript miRNA PCR Arrays and miScript Primer Assays.

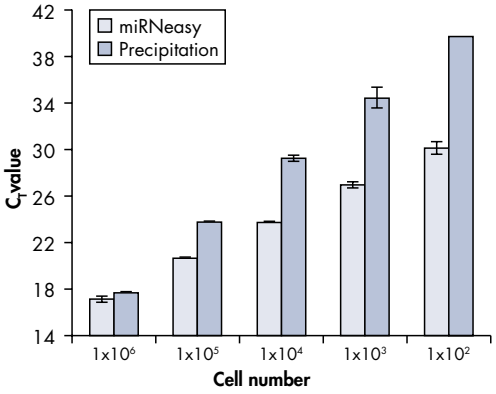


Figure 18. miRNeasy Kit outperforms phenol-based extraction and precipitation. Total RNA including miRNA was purified from a range of amounts of Jurkat cells using the miRNeasy Mini Kit or phenol-based extraction and precipitation. Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16. Results showed that C_T values were lower after purification using the miRNeasy Kit, indicating that higher amounts of miRNA were purified than using phenol-based extraction and precipitation. miRNA was effectively purified from as little as 1 x 10^2 cells using the miRNeasy Kit. In contrast, no miRNA was detected after 40 PCR cycles from 1 x 10^2 cells after phenol-based extraction and precipitation.

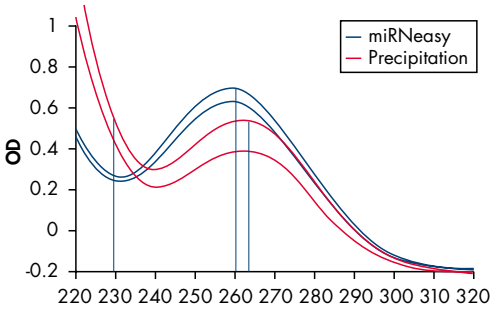


Figure 19. Highly pure RNA without phenol carryover. Total RNA including miRNA was purified from 1 x 10^6 Jurkat cells using the miRNeasy Mini Kit or phenol-based extraction and precipitation. The absorbance spectra showed the OD maximum for RNA purified using the miRNeasy Kit was at 260 nm. In contrast, the OD maximum was greater than 260 nm when precipitation was used for purification, indicating phenol carryover. In addition, the OD_{230} measurement was higher in the precipitation-prepared RNA, indicating salt carryover.

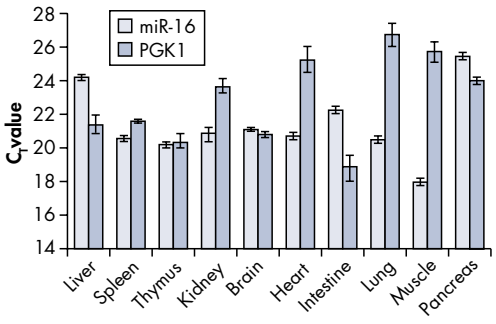


Figure 20. Efficient copurification from a wide range of tissues. Total RNA, including miRNA, was purified from 25 mg of a range of RNA*later*® stabilized rat tissues using the miRNeasy 96 Kit. Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16 and for the larger mRNA of the PGK1 gene. Results showed successful detection of both PGK1 mRNA and miR-16 from the same eluates.

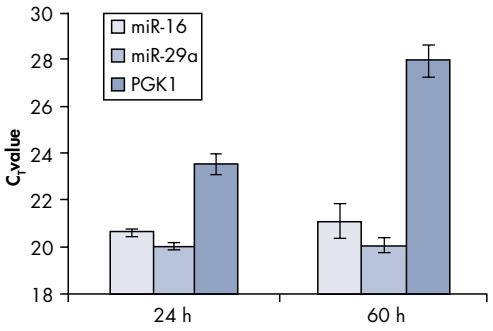


Figure 21. Effective real-time RT-PCR quantification of miRNA from FFPE tissues Rat liver tissue was formalin fixed for 24 hours or 60 hours, followed by purification of total RNA including miRNA using the miRNeasy FFPE Kit. Purified RNA was used as a template in quantitative, real-time RT-PCR using the miScript PCR System to detect miRNAs miR-16 and miR-29a and for the larger mRNA of the PGK1 gene. Evidently, prolonged fixation negatively affects RNA integrity, especially of longer PCR amplicons. Results show successful detection of both miRNAs as well as for the mRNA from the same eluate.

Effective purification from cells and tissues

miRNeasy Mini, Micro, and 96 Kits efficiently purify RNA from tissues and cells, even when low amounts of starting material are used. RNA can be purified from a variety of tissues and cells, including difficult-to-lyse tissues. Purification of total RNA, including miRNA, allows direct comparison of miRNA expression levels with those of target mRNAs, housekeeping reference genes, or any other mRNA of interest (Figure 20).

Circulating miRNA purification from serum or plasma

Circulating miRNAs could serve as potential biomarkers for the detection of different cancers and other diseases, such as diabetes. Using RNA purified from serum or plasma, unique expression of several miRNAs has been shown for several cancers. The miRNeasy Serum/Plasma Kit enables total RNA purification from small volumes of plasma or serum. The miRNeasy Serum/Plasma Spike-In Control provides a synthetic spike-in control for normalization when working with serum or plasma samples. Purified RNA can then be used for biomarker discovery with the miScript PCR System.

High-purity RNA from FFPE sections

Crosslinking and fragmentation can make purification of nucleic acids from FFPE sections challenging. The miRNeasy FFPE Kit provides special lysis and incubation conditions to reverse formalin crosslinking of RNA and efficiently purify RNA while avoiding further RNA degradation. To remove even trace amounts of small DNA fragments, which can impair downstream assays such as real-time RT-PCR, RNA is treated with DNase and DNase Booster Buffer, which optimizes the DNase reaction. Total RNA prepared using the miRNeasy FFPE Kit can then be used with the miScript PCR System to unlock valuable expression data (Figure 21 and Figure 6, page 7).

Ordering Information

Product	Contents	Cat. no.
miRNeasy Mini Kit (50)	Columns, plasticware, and reagents for 50 preps	217004
miRNeasy Micro Kit (50)	Columns, plasticware, and reagents for 50 preps	217084
miRNeasy Serum/Plasma Kit (50)	Columns, plasticware, and reagents for 50 preps	217184
miRNeasy Serum/Plasma Spike-In Control	10 pmol *C. elegans* miR-39 miRNA mimic spike-in control for serum/plasma samples	219610
miRNeasy 96 Kit (4)	Columns, plasticware, and reagents for 4 x 96 preps	217061
miRNeasy FFPE Kit (50)	Columns, plasticware, and reagents for 50 preps	217504
PAXgene Tissue miRNA Kit (50)	Columns, plasticware, and reagents for 50 preps	766134
PAXgene Blood miRNA Kit (50)	Columns, plasticware, and reagents for 50 preps	763134
QIAsymphony PAXgene Blood RNA Kit (96)	Reagent cartridges, accessories, and buffers for 96 preps	762635
miScript II RT Kit (12)	Reagents for 12 x 20 µl cDNA synthesis reactions	218160
miScript II RT Kit (50)	Reagents for 50 x 20 µl cDNA synthesis reactions	218161
miScript SYBR Green PCR Kit (200)	Reagents for 200 x 50 µl PCRs	218073
miScript SYBR Green PCR Kit (1000)	Reagents for 1000 x 50 µl PCRs	218075
miScript PCR Starter Kit (80)	Reagents for 12 x 20 µl cDNA synthesis reactions and 80 x 25 µl PCRs	218193
miScript Primer Assay (100)	miRNA-specific primer for 100 x 50 µl PCRs	Varies*
miScript Precursor Assay (100)	Precursor-specific primer pair for 100 x 50 µl PCRs	Varies*
miRNome miScript miRNA PCR Array	miRNome panels of miRNA assays	331222
miScript miRNA PCR Array	Pathway or disease panels of miRNA assays	331221
Custom miScript miRNA PCR Array	Custom panels of miRNA assays	331231
miScript miRNA Mimic	1 nmol, 5 nmol, or 20 nmol mimic	Varies*
miScript miRNA Inhibitor	1 nmol, 5 nmol, or 20 nmol inhibitor (option of phosphorothioate modification)	Varies*
miScript Target Protector	5 nmol target protector	Varies*

* Visit GeneGlobe to search for and order these products (www.qiagen.com/GeneGlobe).

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China ▪ Telephone 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325 or 400-880-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

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