qScript® microRNA cDNA Synthesis Kit

Cat. No 95107-025 Size: 25 x 20 µL reactions **Store at -25°C to -15°C**
95091-025 100 x 20 µL reactions

MicroRNAs (miRNAs) are small (approximately 22-24 nucleotide) non-coding RNA molecules that are important regulators of gene expression in a variety of organisms. The distinct expression patterns of miRNAs in different tissues, cell development stages, and disease models suggest that miRNAs play important roles in biological processes. The qScript™ microRNA cDNA Synthesis Kit provides a convenient, reliable, and highly sensitive system for conversion of miRNAs into cDNA from total RNA samples. The kit is used in concert with custom miRNAs and the PerfeCTa SYBR® Green SuperMix family of qPCR products as part of the qScript microRNA Quantification System providing a highly sensitive method for quantification and profiling of miRNAs.

Description

The qScript microRNA cDNA Synthesis Kit has been designed to convert miRNAs into cDNA starting from total RNA or RNA preparations pre-enriched for miRNAs. MicroRNAs are not polyadenylated in nature. With the qScript microRNA cDNA Synthesis Kit miRNAs are polyadenylated in a poly(A) polymerase reaction. qScript Reverse Transcriptase and other necessary reagents for cDNA synthesis are subsequently added to convert the poly(A) tailed miRNAs into cDNA using an oligo-dT adapter primer. The adapter primer has a unique sequence at its 5' end which allows amplification of cDNAs in real-time qRT-PCR reactions.

The kit includes a Human Positive Control Primer that can be used to quantify the small nucleolar RNA SNORD44 which is ubiquitously expressed in most human tissues. In addition, the kit contains 20% extra Poly(A) Tailing Buffer and microRNA cDNA Reaction Mix to accommodate the use of no poly(A) polymerase and no reverse transcriptase control reactions.

Individual miRNAs are quantified in real-time SYBR Green qRT-PCR amplification reactions with the desired customer miRNAs and the PerfeCTa Universal PCR Primer (specific to the unique sequence of the oligo-dT adapter primer). The custom miRNAs provide maximum sensitivity and specificity in qRT-PCR amplification and quantification of microRNAs.

Primer design guidelines for custom miRNAs are provided here: https://www.quantabio.com/products/microrna-profiling

Components

	95107-025	95107-100
Poly(A) Tailing Buffer (5X)	1 x 60 µL	1 x 240 µL
Poly(A) Polymerase	1 x 25 µL	1 x 100 µL
microRNA cDNA Reaction Mix	1 x 270 µL	1 x 1080 µL
qScript Reverse Transcriptase	1 x 25 µL	1 x 100 µL
PerfeCTa Universal PCR Primer	1 x 125 µL	1 x 500 µL
PerfeCTa Human Positive Control Primer	1 x 25 µL	1 x 100 µL
Nuclease-Free Water	1 x 1.5 mL	1 x 1.5 mL

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Reaction Protocol

Poly(A) Tailing Reaction

1. Thaw all components (except enzyme), mix thoroughly, and centrifuge briefly. Keep on ice before use.
2. Add the following components to a 0.2 mL PCR tube or 96-well plate on ice:

Component	Volume
Poly(A) Tailing Buffer (5X)	2 µL
RNA (up to 1 ug total RNA or miRNA-enriched RNA)	up to 7 µL
Nuclease-Free Water	variable
Poly(A) Polymerase	1 µL
Final Volume	10 µL

3. Mix components by gentle vortexing and centrifuge briefly to collect the contents.
4. Incubate 60 minutes at 37°C followed by 5 minutes at 70°C. Briefly centrifuge to collect the contents. Keep on ice before cDNA synthesis. The incubation at 37°C can be shortened to 20 minutes when using 100 ng or less of total RNA.

First-Strand cDNA Synthesis Reaction

5. Setup the cDNA Synthesis Reaction as follows:

Component	Volume
Poly(A) Tailing Reaction (from step 4 above)	10 µL
microRNA cDNA Reaction Mix	9 µL
qScript Reverse Transcriptase	1 µL
Final Volume	20 µL

6. Mix by gentle vortexing and then briefly centrifuge to collect contents.
7. Incubate 20 minutes at 42°C followed by 5 minutes at 85°C.

cDNA is now ready to use for qRT-PCR. If desired, the microRNA cDNA product can be diluted with 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA. Diluted cDNA is stable for several months at +4°C. For long-term storage store the cDNA at –20°C.

Real-Time SYBR Green qRT-PCR Amplification of MicroRNAs

Real-time SYBR Green qRT-PCR is performed using 200 nM of each PerfeCTa microRNA Assay Primer and PerfeCTa Universal PCR Primer along with the appropriate PerfeCTa SYBR Green SuperMix product depending on the instrument platform being used.

For each qRT-PCR reaction add the following components:

Component	Volume
PerfeCTa SYBR Green SuperMix (2X)	25 µL
Custom miRNA Assay Primer (10 µM)	1.0 µL
PerfeCTa Universal PCR Primer (10 µM)	1.0 µL
MicroRNA cDNA	up to 23 µL
Nuclease-Free Water	variable
Final Volume	50 µL

The amount of microRNA cDNA can be varied depending on the expression level of the miRNA. As a starting point use about 1ng of total RNA equivalent per qRT-PCR reaction. For miRNAs expressed at low levels you may use 10 ng of total RNA equivalent per qRT-PCR reaction. For most applications 20 to 25 µL qRT-PCR reaction volumes are suitable but reaction volumes can be scaled according to your needs.

2-Step Cycling Protocol

Pre-incubation/activation:	95°C, 2 minutes
PCR (40 cycles)	
Denature:	95°C, 5 seconds
Anneal:	60°C, 30 seconds (collect fluorescence data)

3-Step Cycling Protocol (Optional)

Pre-incubation/activation:	95°C, 2 minutes
PCR (40 cycles)	
Denature:	95°C, 5 seconds
Anneal:	60°C, 15 seconds
Extend:	70°C, 15 seconds (collect fluorescence data)

Use of a 3-step cycling protocol or slightly higher annealing temperature may improve the specificity of some assays. Melt curve analysis is optional. Most microRNA qRT-PCR reactions will produce a single, slightly broader first-derivative melt peak compared to reactions using two gene-specific primers due to slight heterogeneity in the poly(A) tail length.

Relevant Products

- Custom miRNAs: (https://www.quantabio.com/products/microrna-profiling)
- PerfeCTa SYBR Green SuperMix family of qPCR products (www.quantabio.com/group/qpcr_sybr.php)

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