PerfeCTa® Universal PCR Primer

Cat No: 95109-500 Size: 500 x 50 µL reactions **Store at +4°C**

Description

The PerfeCTa® Universal PCR Primer has been designed and validated to work specifically with PerfeCTa microRNA Assays and PerfeCTa SYBR Green SuperMix using first-strand cDNA produced with the qScript microRNA cDNA Synthesis Kit as template in real-time SYBR Green qRT-PCR amplification reactions. Together these products make up the qScript microRNA Quantification System providing a highly sensitive method for quantification and profiling of microRNAs (miRNAs).

The PerfeCTa® Universal PCR Primer is homologous to a unique sequence at the 5'-end of the oligo-dT adapter primer used for cDNA synthesis as part of the qScript microRNA cDNA Synthesis Kit. miRNAs are not polyadenylated in nature. With the qScript microRNA cDNA Synthesis Kit miRNAs are first polyadenylated in a poly(A) polymerase reaction (Figure 1, Step 1). 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 (Figure 1, Step 2). The unique sequence at the 5' end of the adapter primer allows amplification of microRNA cDNAs in real-time SYBR Green qRT-PCR reactions using the PerfecTa Universal PCR Primer and individual PerfecTa microRNA assays (Figure 1, Step 3). The pre-designed and validated PerfeCTa microRNA Assays provide maximum sensitivity and specificity in qRT-PCR amplification and quantification of microRNAs.

For a complete list of available microRNA assays please visit www.quantabio.com/microrna.

Component	95109-500
PerfeCTa Universal PCR Primer	1 x 500 µL

Storage and Stability

The PerfeCTa Universal PCR Primer is stable for > one year when stored at +4°C.

Real-Time SYBR Green qRT-PCR Amplification of MicoRNAs

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
SYBR Green SuperMix (2X)	25 µL
PerfeCTa microRNA 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 1 ng 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.

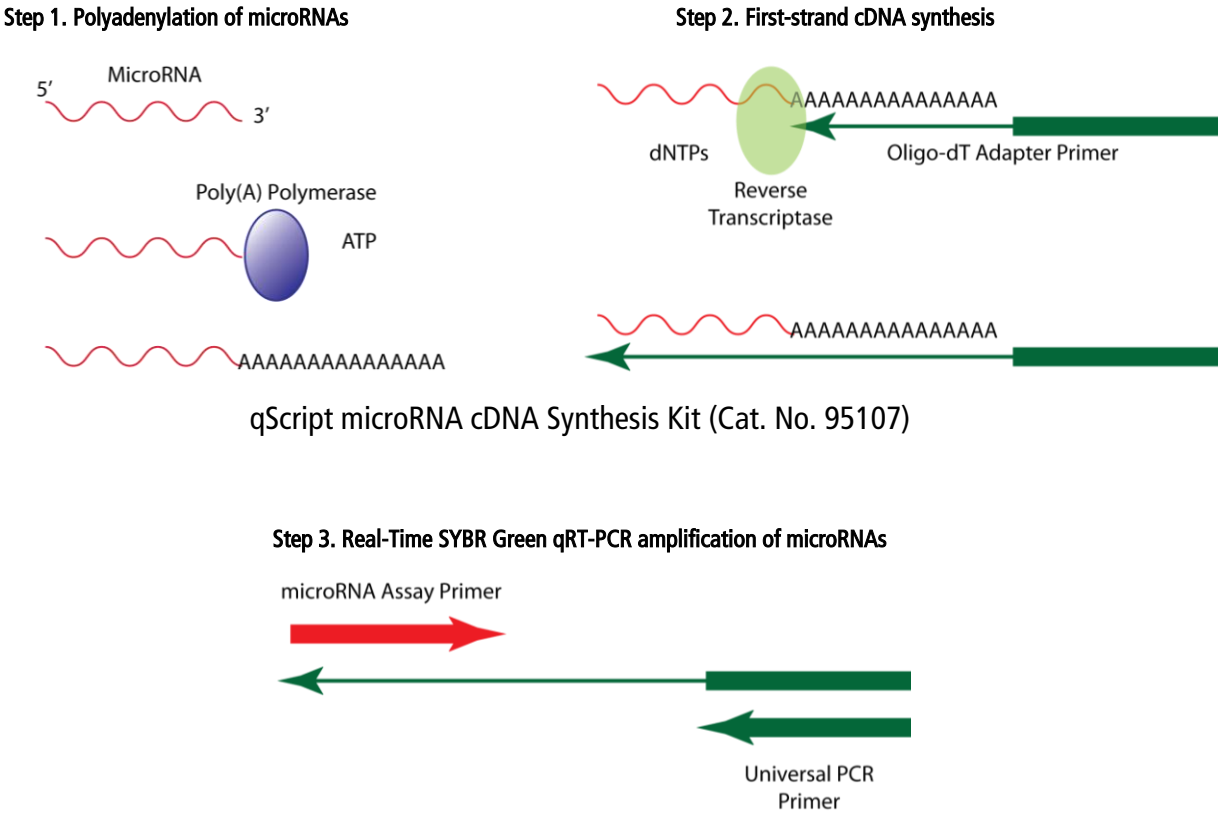

Figure 1. MicroRNA analysis using the qScript microRNA Quantification System.

With the qScript microRNA cDNA Synthesis Kit miRNAs are first polyadenylated in a poly(A) polymerase reaction (**Step 1**). qScript Reverse Transcriptase and other necessary reagents for cDNA synthesis are subsequently added to convert the poly(A) tailed miRNAs into first-strand cDNA using an oligo-dT adapter primer (**Step 2**). The unique sequence at the 5′ end of the adapter primer allows amplification of microRNA cDNAs in real-time SYBR Green qRT-PCR reactions using the PerfeCTa Universal PCR Primer and individual PerfeCTa microRNA assays (**Step 3**).

Relevant Products

- qScript microRNA cDNA Synthesis Kit (Cat. No. 95107)
- PerfeCTa microRNA Assays (www.quantabio.com/microrna)
- PerfeCTa SYBR Green SuperMix family of qPCR products (www.quantabio.com/group/qpcr_sybr.php)

Precautions and Disclaimer

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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