qScript™ cDNA Synthesis Kit

Cat. No.	95047-025	Size:	25 x 20-µL reactions	**Store at -20°C**
	95047-100		100 x 20-µL reactions	
	95047-500		500 x 20-µL reactions	

Description

The qScript cDNA Synthesis Kit is a sensitive and easy-to-use solution for RNA quantification using two-step RT-PCR. The novel qScript Reaction Mix provides all the necessary components for cDNA synthesis except enzyme and RNA template. The optimized blend of random and oligo(dT) primers provides robust, consistent and unbiased first-strand synthesis over a broad range of RNA template concentrations. qScript reverse transcriptase is a mixture of an engineered MMLV RT and a ribonuclease inhibitor protein. The simplified reaction procedure is ideally suited for high throughput expression studies using real-time quantitative RT-PCR. The resulting cDNA product is directly compatible with current real-time PCR methods or conventional end-point RT-PCR of targets ≤1 kb in length.

Components

	95047-025	95047-100	95047-500
qScript Reaction Mix (5X) 5X concentrated solution of optimized buffer, magnesium, oligo(dT) and random primers, and dNTPs	1 x 100 µL	1 x 400 µL	2 x 1 mL
qScript Reverse Transcriptase, 20X concentration	1 x 25 µL	1 x 100 µL	1 x 500 µL
Nuclease-free water	1 x 1.5 mL	1 x 1.5 mL	4 x 1.5 mL

Storage and Stability

Kit components are stable for 2 years when stored in a constant temperature freezer at -20°C. After thawing, mix thoroughly before using.

Reaction Protocol

1. Thaw all frozen components. Mix thoroughly, and briefly centrifuge to collect contents before using. Place all components, including qScript RT on ice.
2. Add the following to a 0.2-mL thin-walled PCR tube or 96-well PCR reaction plate sitting on ice:

RNA (1 µg to 10 pg total RNA)	variable
Nuclease-free water	variable
qScript Reaction Mix (5X)	4.0 µL
qScript RT	1.0 µL
final volume	20.0 µL

Note: When performing multiple first-strand reactions, a master mix can be prepared with water, qScript Reaction Mix and qScript RT.

	Single rxn.	25 rxns.	100 rxns.
Nuclease-free water	10 µL	250 µL	1000 µL
qScript Reaction Mix (5X)	4 µL	100 µL	400 µL
qScript RT	1 µL	25 µL	100 µL
total volume	15 µL	375 µL	1500 µL

Dispense 15 µL of cDNA master mix to each well / tube.
Add 5 µL of RNA sample to each reaction.
Cover the reaction plate with sealing film or cap each reaction.

3. Vortex gently, and then centrifuge 10s to collect contents.
4. Place tube(s) in a thermal cycler programmed as follows:
   - 1 cycle: 22°C, 5 min
   - 1 cycle: 42°C, 30 min
   - 1 cycle: 85°C, 5 min
   - 4°C hold
5. Initiate run.
6. After completion of cDNA synthesis, use 1/5th to 1/10th of the first-strand reaction (2-4 µL) for PCR amplification. If desired, cDNA product can be diluted with 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA and stored at -20°C.

Guidelines for Reverse Transcription-qPCR

Minus RT-controls: Accurate quantification of gene expression by RT-qPCR requires testing and reporting the extent of contamination of genomic DNA in each RNA sample for each gene of interest. The presence of trace amounts of gDNA does not usually interfere with quantification of high copy reference genes. However, it can have a significant contribution on signal for low copy genes. Even when using primers that are separated by intronic sequence or bridge exon junctions, the presence of genomic DNA can produce positive signals from amplification of pseudogene or off-target PCR product. Therefore, it is important to always include the appropriate "no RT" or "minus RT" control reactions in your experimental design.

While it is feasible to construct a formal cDNA synthesis control that includes all components except the RT, the most direct method to test for the presence of genomic DNA is to bypass the RT step and use an equivalent amount of the RNA preparation directly for PCR amplification. For example: if you start with 1 µg of total RNA for cDNA synthesis and use 1/10th of the first-strand reaction as template for qPCR; then use 100 ng of total RNA as template for the minus RT-control qPCR. Any signal from the RNA only reaction is attributable to the presence of genomic DNA.

DNase digestion of total RNA: Trace levels of genomic DNA can obscure accurate quantification, particularly when the specific gene(s) of interest are low copy. PerfeCta® DNase I is a high purity, recombinant DNase I preparation that is free of any contaminating RNases. It provides a simple and rapid solution to eliminate residual genomic DNA that is directly compatible with qScript cDNA SuperMix, or other first-strand synthesis kits. The supplied Reaction Buffer and proprietary Stop Buffer support a simple heat-kill step that permanently inactivates all trace levels of DNase activity before the cDNA synthesis step. Heat-kill procedures used by other DNase I reagents are ineffective and not compatible with qScript cDNA SuperMix. Residual, or renatured, DNase will degrade cDNA product and alter apparent expression levels. If using other sources of RNase-free DNase I, it is essential to remove all traces of DNase activity before proceeding with first-strand synthesis. Suitable RNA purification methods include phenol:chloroform extraction followed by ethanol precipitation, or the use of chaotropic salts and a silica-based RNA purification cartridge or column. Please call technical support at (800) 364-2149 or visit our web site at www.quantabio.com if you require additional information or protocols.

Quality Control

Kit components are free of contaminating DNase and RNase. The qScript cDNA Synthesis Kit is functionally tested in reverse transcription quantitative PCR (RT-qPCR). First-strand synthesis is performed in triplicate on each dilution of a log-fold serial dilution of HeLa cell total RNA from 1 pg to 1 µg. One-tenth of each first-strand reaction is used for qPCR amplification. Kinetic analysis must demonstrate linear resolution over five orders of dynamic range ($r2 > 0.995$) and a PCR efficiency > 90%.

Limited Use Label License

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Related Products

PerfeCta DNase I, Cat. No. 95150-01K

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95047 REV 150319