

## qScript™ cDNA SuperMix

Cat No.	95048-025	Size:	25 x 20- $\mu$ L reactions	(1 x 100 $\mu$ L)	<b>Store at -20°C</b>
	95048-100		100 x 20- $\mu$ L reactions	(1 x 400 $\mu$ L)	
	95048-500		500 x 20- $\mu$ L reactions	(2 x 1 mL)	
	95048-096		480 x 20- $\mu$ L reactions	(5 x 96 well plates)	

### Description

qScript cDNA SuperMix provides a sensitive and easy-to-use solution for two-step RT-PCR. This 5X concentrated master mix provides all necessary components (except RNA template) for first-strand synthesis including: buffer, dNTPs, MgCl<sub>2</sub>, primers, RNase inhibitor protein, qScript reverse transcriptase and stabilizers. qScript is a RNase H(+) derivative of MMLV reverse transcriptase, optimized for reliable cDNA synthesis over a wide dynamic range of input RNA. The unique blend of oligo (dT) and random primers in the qScript cDNA SuperMix works exceptionally well with a wide variety of targets. This blend is optimized for the production of targets < 1 kb in length. qScript cDNA SuperMix produces excellent results in both real-time and conventional RT-PCR.

### Components

qScript cDNA SuperMix 5X reaction buffer containing optimized concentrations of MgCl<sub>2</sub>, dNTPs (dATP, dCTP, dGTP, dTTP), recombinant RNase inhibitor protein, qScript reverse transcriptase, random primers, oligo(dT) primer and stabilizers.

### Storage and Stability

qScript cDNA SuperMix is stable for 1 year when stored in a constant temperature freezer at -20°C. To extend the product's shelf-life, store the kit at -70°C. qScript cDNA SuperMix showed no loss in functional performance after 20 cycles of freezing on dry ice and thawing on ice. However, we recommend that the number of freeze-thaw cycles be kept to a minimum.

### Reaction Assembly

Place components on ice. Mix, and then briefly centrifuge to collect contents to the bottom of the tube before using.

Component	Volume for 20- $\mu$ L rxn.	Final Concentration
qScript cDNA SuperMix (5X)	4 $\mu$ L	1X
RNA template	variable	(1 $\mu$ g to 10 $\mu$ g total RNA)
RNase/DNase-free water	<u>variable</u>	
Total Volume ( $\mu$ L)	20 $\mu$ L	

**Note:** for smaller reaction volumes (i.e. 10- $\mu$ L reactions), scale components proportionally.

### Reaction Protocol

- Combine reagents in 0.2-mL micro-tubes or 96-well plate sitting on ice.
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.
- Incubate:
  - 5 minutes at 25°C
  - 30 minutes at 42°C
  - 5 minutes at 85°C
  - Hold at 4°C
- After completion of cDNA synthesis, use 1/5th to 1/10th of the first-strand reaction (2-4  $\mu$ L) 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.

Since the reverse transcriptase is an integral component of qScript cDNA SuperMix, it is not 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 1/10<sup>th</sup> 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:* If trace levels of genomic DNA obscure accurate quantification of your gene(s) of interest, use a high quality, RNase-free preparation of DNase I to remove residual genomic DNA. After the DNase digestion, 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. Simple "heat-kill" procedures or the use of inactivating slurry solutions are not compatible with qScript cDNA SuperMix. Please call technical support at (800) 364-2149 or visit our web site at [www.quantabio.com](http://www.quantabio.com) if you require additional information or protocols.

## Quality Control

Kit components are free of contaminating DNase and RNase. qScript cDNA SuperMix 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 ( $r^2 > 0.995$ ) and a PCR efficiency  $> 90\%$ .

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