Quanta BIOSCIENCES™

qScript™ XLT cDNA SuperMix

Cat No.	95161-025	Size:	25 x 20-µL reactions	(1 x 100 µL)	Store at -20ºC
	95161-100		100 x 20-µL reactions	(1 x 400 µL)	
	95161-500		500 x 20-µL reactions	(2 x 1 mL)	

Description

qScript XLT cDNA SuperMix is a next-generation tool for first-strand cDNA synthesis, providing a highly sensitive and easy-to-use solution for two-step RT-PCR and RT-qPCR. qScript XLT is an engineered M-MLV reverse transcriptase with reduced RNase H activity and improved activity and stability at higher temperatures. Combined with a precise mixture of reaction components, this SuperMix enables superior results over a wide dynamic range of input RNA, with up to 8-fold higher sensitivity than cDNA synthesis kits utilizing an RNase H(+) reverse transcriptase (RT). This 5X concentrated master mix provides all necessary components (except RNA template) for first-strand synthesis including: buffer, dNTPs, $MgCl_2$, primers, RNase inhibitor protein, qScript XLT reverse transcriptase and stabilizers. The unique blend of oligo (dT) and random primers in the qScript XLT cDNA SuperMix captures unbiased representation of all RNA sequences into cDNA product (including the 5'-end, 3'-end, or central regions of long RNAs) and works exceptionally well with a wide variety of RNA templates. However, due to the relatively short average length of random-primed first-strand product, amplicon length for conventional RT-PCR applications should be limited to 1 kb or less.

Components

qScript XLT cDNA SuperMix	5X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), recombinant RNase inhibitor protein, qScript reverse transcriptase, random primers, oligo(dT) primer and stabilizers.

Storage and Stability

qScript XLT cDNA SuperMix is stable for 1 year when stored in a constant temperature freezer at -20°C. The kit may be stored at -80°C to extend the product's shelf life. qScript XLT cDNA SuperMix showed no loss in functional performance after 20 cycles of freezing on dry ice and thawing on ice.

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-µL rxn.	Final Concentration
qScript XLT cDNA SuperMix (5X)	4 µL	1X
RNA template	variable	(2 µg to 10 pg total RNA)
RNase/DNase-free water	variable	
Total Volume (µL)	20 µL	

Note: for smaller reaction volumes (i.e. 10-µL 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 25ºC
  60 minutes at 42ºC*
  5 minutes at 85ºC
  Hold at 4ºC

  *Note: These conditions are provided as a general guideline and support maximum cDNA yield and sensitivity for global gene expression profiling. Specific applications may benefit from modified reaction conditions. qScript XLT can be used at temperatures up to 54°C for RNA with stable secondary structure(s). However, higher temperatures can compromise cDNA yield for other RNAs. Shorter incubation times (30 min) can be applied when using <500 ng of total RNA template without compromising cDNA yield.

- Use 1/10th (or less) of the first-strand product as template for PCR / qPCR amplification. The optimal amount of cDNA for PCR can vary depending on the amount of starting RNA template, choice of detection chemistry, and abundance of the specific target sequence. The high yield of cDNA resulting from reactions containing ≥ 500 ng of total RNA can overwhelm detection of specific product by SYBR® Green I qPCR. If required, dilute cDNA product with 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA. Store first-strand product at -20ºC.

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 XLT 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 1/10th 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 XLT 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 XLT 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. qScript XLT 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 ($r^2 > 0.990$) and a PCR efficiency > 90%.

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Related Products

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

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