



qScript® Ultra SuperMix

Cat No.	95217-025	Size:	25 x 20- μ L reactions
	95217-100		100 x 20- μ L reactions
	95217-500		500 x 20- μ L reactions

Store at -25°C to -15°C

Description

The qScript Ultra SuperMix is a convenient, rapid, easy to use first-strand cDNA synthesis system for RT-PCR and RT-qPCR applications that demand the highest consistency, reproducibility, and unbiased representation of the transcriptome in cDNA product. Incorporating an optimized mixture of both anchored oligo(dT) and random primers, the highly stabilized 5X-concentrated qScript Ultra SuperMix contains all required components for cDNA synthesis except RNA template. This simplifies reaction assembly and improves precision of technical replicate reactions.

A key component is the qScript Ultra reverse transcriptase (RT). This novel, engineered, RNase H deficient RT sets a new standard for cDNA synthesis with increased thermostability, velocity, processivity, and resistance to many common reaction inhibitors. The superior performance of this novel RT is further supported by proprietary replication accessory proteins and a recombinant mammalian RNase inhibitor protein. These features allow for reactions to be carried out at higher temperatures than other engineered retroviral RTs, improving sensitivity and minimizing potential interference from RNA with high GC content or stable secondary structures. The improved speed, processivity, and expanded thermal activity profile of qScript Ultra provides linear conversion of RNA to cDNA from 2.5 μ g to 1 μ g of total RNA in a rapid 10-minute reaction at 55°C.

qScript Ultra SuperMix is compatible with total RNA, polyA+ RNA, or viral RNA and includes a non-interfering blue tracking dye that facilitates visualization and precision during manual or automated reaction assembly. The system delivers high and consistent conversion efficiencies enabling reliable RT-qPCR of challenging RNAs, including low abundance transcripts or low copy viral RNA, even in the presence of common reaction inhibitors or high levels of carrier RNA. The resulting cDNA product is directly compatible with real-time RT-qPCR methods or end-point RT-PCR of amplicons \leq 300 nucleotides in length. For applications requiring longer first-strand products, the qScript Ultra Flex kit (catalog number 95215) is recommended.

Components

	95217-025	95217-100	95217-500
Component Description			
5x qScript Ultra SuperMix	1 x 100 μ l	1 x 400 μ l	1 x 2 mL
Optimized master mix containing buffer, magnesium, dNTPs, optimized primer mix and qScript Ultra RT			

Storage and Stability

Store the product in a constant temperature freezer at -25°C to -15°C upon receipt.

After thawing, mix thoroughly before use.

For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Standard Reaction Assembly

1. Thaw components, mix and centrifuge before use. Hold on ice before use.
2. Add the following to a thin-walled PCR tube or reaction plate on ice:

Component	Volume for 20 μ L rxn.	Final Concentration
Nuclease-free water	variable	
Template RNA	variable	2.5 μ g to 1 pg total RNA
5X qScript Ultra SuperMix	4 μ l	
Final volume	20 μ l	

Note: for multiple reactions, a master mix can be prepared with all components except template RNA and dispensed into 96-well plates or PCR tubes.

3. Mix by gentle vortexing, then briefly centrifuge to collect contents.
4. Incubate for:
 - o 2 minutes at 25°C
 - o 10 minutes at 55°C
 - o 1 min at 95°C
 - o Hold at 4°C
5. After the completion of cDNA synthesis, reactions can be used directly for endpoint RT-PCR or RT-qPCR analysis. It is recommended that PCR reactions contain no more than 1/5 volume of the first-strand cDNA reaction. If desired, reactions can be diluted with TE buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA). Reactions can be stored at -20°C for future use or at -80°C for long-term storage.

No reverse transcription genomic DNA controls

Accurate quantification of gene expression by RT-qPCR requires testing and measuring 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 typically interfere with quantification of high copy reference genes, but can have a significant contribution on the signal detected for low copy genes. A common strategy is to use primers that are separated by intronic sequence or bridge exon-exon junctions, but even in these cases it is possible for the presence of genomic DNA to produce positive signals from amplification of pseudogene or off-target PCR product. Therefore it is important to include appropriate control reactions in the experimental design.

Since the reverse transcriptase is an integral component of the qScript Ultra SuperMix, it is not feasible to construct a formal cDNA synthesis control that includes all components except the RT. Instead, 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 starting with 1 μ g of total RNA for cDNA synthesis and using 1/10th of the first-strand reaction as template for qPCR, then simply use 100 ng of total RNA as template for the minus RT-control qPCR. Any signal from the RNA template reaction is attributable to the presence of genomic DNA.

DNase digestion of total RNA

To remove trace levels of genomic DNA, a high quality RNase-free DNase may be used to treat RNA samples prior to first-strand cDNA synthesis, but care should be taken to ensure that the DNase is completely inactivated after the digestion is complete. This can be accomplished using heat inactivation in the presence of an appropriate chelating “stop” buffer or by using a suitable RNA purification method. Any residual, or renatured DNase will degrade cDNA product and alter apparent expression levels.



The Quantabio Extracta Plus RNA kit (catalog number 95214) provides the rapid purification of high-quality total RNA from cultured cells or tissue and includes a specially designed DNA removal column that effectively eliminates genomic DNA with a simple lysate spin filtration. With this kit there is no need for a separate DNase treatment of the purified RNA template.

Quality Control

Kit components are free of contaminating DNase and RNase. qScript Ultra SuperMix is functionally tested in RT-qPCR for detection of β -actin mRNA in triplicate reactions using log-fold serial dilutions of total RNA from 1 μ g to 1 pg, followed by qPCR amplification using 1/10 of each first-strand reaction. Analysis must demonstrate a coefficient of determination (R^2) \geq 0.990 with a slope analysis between -3.20 and -3.70.

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