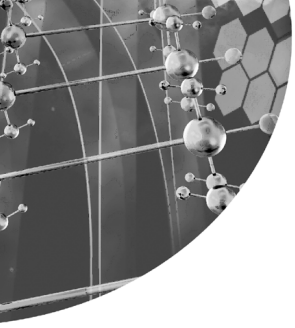

RealMasterScript™ SuperMix Kit

For efficient first strand cDNA synthesis in a convenient master mix format

Product specifications

Product description

RealMasterScript SuperMix Kit is a 5X concentrated ready-to-use master mix which provides all necessary components (expect RNA template) for synthesis of cDNA template for two-step RT-PCR. The RealMasterScript SuperMix Kit is optimized for the production of cDNA targets < 1 kb in length for subsequent qPCR and end-point PCR.

Product limitations

RealMasterScript SuperMix Kit is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

Materials supplied

Kit	Order No	Size
RealMasterScript SuperMix Kit - 25 Rxn	2201020	1 x 100 µl
RealMasterScript SuperMix Kit - 100 Rxn	2201030	1 x 400 µl

RealMasterScript SuperMix contains optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), recombinant RNase inhibitor protein, RealMasterScript Reverse Transcriptase, random primers, oligo(dT) primer and stabilizers.

Storage and stability

The RealMasterScript SuperMix Kit is shipped on dry ice.

RealMasterScript SuperMix Kit is stable until the expiration date indicated on the kit label if it is stored in a constant temperature freezer at -20°C. To extend the product's shelf-life, store the kit at -70°C.

The product retains full functional performance after repeated freeze-thaw cycles.

Safety information

All due care and attention should be exercised in the handling of this product. We recommend all users of 5 PRIME products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines. Specifically, always wear a suitable lab coat, disposable gloves, and protective goggles when working with chemicals.

Neither of the vials contains hazardous substances in reportable quantities. The usual precautions taken when handling chemicals should be observed. Used reagents can be disposed of in waste water in accordance with local regulations. In case of eye contact, flush eyes with water. In case of skin contact, wash off with water. In case of ingestion, seek medical advice.

Additional safety information is available from www.5PRIME.com in material safety data sheets (MSDSs) for 5 PRIME products and 5 PRIME product components.

Quality assurance

5 PRIME products are manufactured using quality chemicals and materials that meet our high standards. All product components are subjected to rigorous quality assurance testing process:

- → **Component testing:** each component is tested to ensure the composition and quality meet stated specifications.
- → **Performance testing:** each product is tested to ensure it meets the stated performance specification.

Additional quality information is available from www.5PRIME.com. Certificate of analysis sheets for 5 PRIME products and 5 PRIME product components can be obtained on request.

Product warranty

5 PRIME is committed to providing products that improve the speed, ease-of-use and quality of enabling technologies. 5 PRIME guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use.

This warranty is in place of any other warranty or guarantee, expressed or implied, instituted by law or otherwise. 5 PRIME provides no other warranties of any kind, expressed or implied, including warranties of merchantability and fitness for a particular purpose. Under no circumstance shall 5 PRIME be responsible for any direct, indirect, consequential or incidental damages or loss arising from the use, misuse, results of use or inability to use its products, even if the possibility of such loss, damage or expense was known by 5 PRIME.

5 PRIME distributors

A complete list of 5 PRIME distributors is available from www.5PRIME.com.

Protocol

Product principle

RealMasterScript SuperMix Kit provides sensitive first-strand cDNA synthesis in an easy-to-use format. The master mix contains all necessary components (except RNA template) for cDNA synthesis

including: buffer, dNTPs, $MgCl_2$, primers, RNase inhibitor protein, RealMasterScript Reverse Transcriptase and stabilizers.

The combination of RealMasterScript (which is a RNase H(+) M-MLV RT) together with the unique blend of oligo (dT) and random primers leads to high cDNA yields for sensitive quantification of even low-abundance transcripts. Furthermore, the primer mix ensures cDNA synthesis from all regions of RNA transcripts (even from 5′ regions) regardless of where the target region is located on the transcript.

Guidelines for Reverse Transcription

RNA integrity, storage and stability: The integrity and quality of template RNA is critical for successful RT-PCR analysis and cDNA synthesis. The RNA must not be degraded by ribonucleases, as indicated by intact ribosomal RNA bands. Chemical impurities, such as protein, poly-anions (e.g., heparin), salts, EDTA, ethanol, and phenol, can affect the activity and processivity of the reverse transcriptase. To ensure reproducible and efficient reverse transcription, it is important to determine the quality and quantity of the starting RNA.

For better results, we recommend starting with RNA purified using 5 PRIME PerfectPure™ RNA Purification System.

Purified RNA may be stored at -20°C or -70°C in water. Under these conditions, no degradation of RNA is detectable after 1 year.

DNA contamination: Contamination of the RNA by even trace amounts of genomic DNA can have a significant contribution on signal, especially for low copy genes. Primers that do not span intron sequences or are designed for genes without introns will amplify PCR products of the same size as the PCR products generated from the specific cDNA target. 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. Residual DNase activity will significantly impair cDNA yield. Due to that we recommend utilizing 5 PRIME PerfectPure System which includes an on column DNase digestion resulting in efficient removal of DNase after DNase digestion.

Generation of minus RT control: Because the reverse transcriptase is an integral component of RealMasterScript SuperMix Kit it is not possible to make 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 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.

Reaction setup

Component	Volume for 20 µl Rxn	Final Concentration
RealMasterScript SuperMix Kit (5X)	4 µl	1X
RNA template	variable	(1 µg to 10 pg total RNA)
RNase/DNase-free water	variable	
Total Volume (µl)	20 µl	

Note: for smaller and higher 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.

Step	Temperature	Time
Primer annealing	25℃	5 minutes
cDNA synthesis	42℃	30 minutes
Inactivation	85℃	5 minutes
Intermediate sample storage	4℃	

After completion of cDNA synthesis, use 1/5 to 1/10 of the first-strand reaction (2-4 µl) for PCR amplification. The optimal input quantity of first-strand product may vary for different RT-qPCR applications and the abundance of the specific target sequence. The use of higher input volumes of first-strand product in the PCR can improve RT-PCR sensitivity for detection of low copy RNAs, or when starting with low input quantities of total RNA (i.e. highly dilute RNA samples). Note that PCR reaction volume and cycling conditions may need to be adjusted to accommodate larger sample volumes. Up to 50% of the first-strand reaction (10 µL) can be used in a 25 µL qPCR with RealMasterMix Fast Probe Kit.

If desired, cDNA product can be diluted with 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA and stored at -20°C.

Limited label licenses