SIGMA-ALDRICH®

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Product Information

ReadyScript™ cDNA Synthesis Mix

Catalog Number **RDRT**
Storage Temperature –20 °C

Technical Bulletin

Product Description
ReadyScript cDNA Synthesis Mix provides a sensitive and easy-to-use solution for two-step RT-PCR. This 5X concentrated master mix provides all necessary components for first-strand synthesis (except RNA template) including: buffer, dNTPs, $MgCl_2$, primers, RNase inhibitor protein, M-MLV reverse transcriptase and stabilizers. The ReadyScript enzyme is an RNAse H (+) modification of M-MLV reverse transcriptase and is optimized for reliable cDNA synthesis over a wide dynamic range of input RNA. The unique blend of oligo (dT) and random primers in the ReadyScript cDNA Synthesis Mix works exceptionally well with a wide variety of targets. This blend is optimized for the production of targets < 1kb in length. ReadyScript cDNA Synthesis Mix produces excellent results in both real-time and conventional RT-PCR

Reagents
Supplied as a 5X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), recombinant RNase inhibitor protein, ReadyScript reverse transcriptase, random primers, oligo(dT) primer and stabilizers.

ReadyScript cDNA Synthesis Mix RDRT-25RXN
RDRT-100RXN
RDRT-500RXN

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Stable for 1 year when stored in a constant temperature freezer at -20 °C. To extend the product's shelf-life, store at -70 °C. ReadyScript cDNA Synthesis Mix showed no loss in functional performance after 20 cycles of freezing on dry ice and thawing on ice. For best performance we recommend that the number of freeze-thaw cycles be kept to a minimum.

Procedure

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

Reagent	Volume for 20 μL reaction	Final Concentration
ReadyScript cDNA Synthesis Mix	4 μL	1X
RNA template	variable	1 μg to 10 pg total RNA
Rnase/DNase-free water	variable	
Total Volume	20 μL	

Note: for smaller or larger reaction volumes, components may be scaled down or up proportionately.

Reaction
- 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 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 quantitation of genomic DNA contamination 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, but 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 ReadyScript cDNA Synthesis Mix, 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: 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 (DNAse I, Catalog Number AMPD1-1KT). 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 (GenElute™ Direct mRNA Miniprep Kit, Catalog Number DMN10). Simple "heat-kill" procedures or the use of inactivating slurry solutions are not compatible with ReadyScript cDNA Synthesis Mix. Please call technical support at 800-325-5832 or visit our web site at www.sigmaaldrich.com if you require additional information or protocols.

Quality Control

Free of contaminating DNase and RNase.

ReadyScript cDNA Synthesis Mix 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%.

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