FIREScript® RT cDNA synthesis MIX

Catalogue Number	Size (20 µl reactions)
06-16-0000S	20 rxn (sample)

Shipping:
At room temperature

Batch Number and Expiry Date:
See vial

Store at –20°C upon receipt

Storage and Stability*:

- Routine storage at –20°C (–28°C to –18°C) until Expiry Date
- Stable at 4°C (2°C to 8°C) for 6 months
- Stable at room temperature (15–25°C) for 1 month
- Freeze-thaw stability: 30 cycles

Reaction setup:
At room temperature

Manufactured by Solis BioDyne, in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

Reagents supplied:

Components
FIREScript® Enzyme Mix
10x RT Reaction Premix without primers (with DTT and dNTPs)
10x RT Reaction Premix with oligo (dT) primer (with DTT, dNTPs, and oligo (dT) primer)
10x RT Reaction Premix with random primers (with DTT, dNTPs, and random primers)
10x RT Reaction Premix with oligo (dT) and random primers (with DTT, dNTPs, oligo (dT) primer, and random primers)
Water, nuclease free

Product description:

- FIREScript® Reverse Transcriptase is a genetically modified MMLV-based robust reverse transcriptase with full RNase H activity. It is an exceptionally stable enzyme due to a unique patented genetic modification in the polypeptide structure called the Stability TAG**.
- The working temperature of enzyme ranges from 37°C – 60°C, with an optimum at 50°C. This proprietary is beneficial for the detection of templates with complicated secondary structures.
- FIREScript® Reverse Transcriptase is a fast enzyme with a reaction time of 5-30 minutes and is able to transcribe up to 8.9 kb RNA sequences.

- Wide range of priming options ensures flexible assay design. FIREScript® Reverse Transcriptase is compatible with oligo (dT), random, and gene-specific primers.

Applications:

- Gene expression analysis
- Disease diagnosis
- Genomic analysis
- Structural studies of mRNA

Step-by-step guidelines:

1. Thaw the reagents at room temperature. Mix each reagent by gentle vortexing or pipetting up and down, then centrifuge briefly.
2. Mix the following components.

Component	Volume	Final conc.
Gene specific primer[1,2]	Variable	0.1–1 µM[3]
10x RT Reaction Premix *according to primer choice*	2 µl	1x
FIREScript® Enzyme Mix	1.5 µl	10 U/µl
Template RNA	0.1–5 µg	Variable
Nuclease-free water	up to 20 µl	
Total	20 µl	

[1] Add gene specific primer ONLY if using 10x RT Reaction Premix without primers.

[2] Not included in FIREScript® cDNA synthesis MIX. Supplied by the end-user.

[3] Titrate the primer concentration to determine the optimal concentration.

3. Incubate the reaction mixture as follows:

Step	Temperature	Time
Primer extension (ONLY if using Reaction premix with random primers or random and oligo(dT) primers)	25°C	5–10 min
Reverse transcription	50°C	5 min[1]
Enzyme inactivation	85°C	5 min

[1] 5 min is sufficient for the synthesis of 1 kb amplicon. Optimize the reverse transcription step according to the troubleshooting guide.

Priming options:

- Oligo(dT) primers:
  - Specifically binds poly(A) tail of a eucaryotic mRNA (the total RNA pool has 1–2% of poly(A)+ RNA)
  - Generates full-length cDNA
- Random primers:
  - Binds at different sites along an RNA molecule
  - Does not depend on the presence of poly(A) tail
- Gene-specific primer:
  - Specific to the gene of interest
  - Increases assay specificity
  - Designing gene-specific primers complementary to the 3' end of the target

Considerations while working with RNA:

- Avoid RNase contamination:
  - Wear protective clothing, gloves, *etc.*
  - Use sterile and RNase-free disposables

- Prior experiment store RNA samples at -70°C or below
- Thaw and keep RNA samples on ice during the experiment

IMPORTANT:
cDNA synthesis reaction mixture components may inhibit PCR reaction. Don't use more than 10% of the cDNA reaction mixture in a final PCR/qPCR reaction.

Troubleshooting guide

Low or no yield:

- Reaction conditions are not optimal – determine the optimal temperature by running a temperature gradient of 37–60°C (for example 37, 42, 50, 55, 60°C) for 15 min. When the optimal temperature is identified the optimal reaction time can be tested (5, 15, 30, and 60 min).
- Poor quality of template – check the template's purity and integrity. A260/280 and A260/230 ratios should be equal to or higher than 1.8 and 2.0 respectively.
- Gene-specific primer design and/or concentration is not optimal – titrate primer concentration (final concentration range between 0.1–1 µM). Use dedicated software, such as open-source Primer3 and NCBI Primer-BLAST to design target-specific primers.
- Reaction components are degraded – check the storage conditions and expiry date of the reagents; perform a positive control with template and reagents previously confirmed to amplify.

- Inhibition of PCR/qPCR reaction - prepare a 5-fold dilution of the cDNA reaction mixture. Use up to 10% of the cDNA reaction mixture in a final (q)PCR reaction.

Truncated cDNA:

- Poor quality of template - check the template's purity and integrity. Control for the presence of inhibitors, contaminants, and degraded template RNA (old samples, degraded while purification, wrong storage conditions, *etc*).
- Sample has a high secondary structure or GC content – preincubate RNA at 65°C for 5 min, then chill on ice for 15 min.

False positive result:

- Poor quality of template - genomic DNA contamination. To prevent amplification from genomic DNA, design primers spanning exon-exon junctions of the target mRNA, or primers that hybridize with sequences in consecutive exons flanking a long (*e.g.* 1 kb) intron. Revise sample preparation step. DNase treatment can prevent genomic DNA contamination.
- Components or disposables are contaminated – check the storage conditions of the reagents; perform a negative control with no template.
- Reaction mixture composition is not optimal - reduce the amount of DNA polymerase by 10%.

Source:
Purified from an *E. coli* strain that carries an overproducing plasmid containing a *FIREScript Reverse Transcriptase* gene.

Unit definition:

One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into an acid-precipitable material in 10 minutes at 37°C using poly(rA)•oligo(dT) as a template in a total reaction volume of 50 µl.

Safety precautions:
Please refer to Safety Data Sheet for more information.

Technical support:

Contact your sales representative for any questions or send an email to support@solisbiodyne.com

For research use only. Not for use in diagnostic procedures.

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*__Product stability__ is assessed using routine QC assays and QC criteria set forth in the product specification and are intended to provide guidelines for shipping and storage conditions only. Customer or its designee shall be responsible for conducting all necessary stability testing applicable to their assay and/or QC criteria, and to comply with any applicable regulatory requirements or guidelines. Such stability testing shall include testing to validate the lead times for shipment, the shelf life of, and the product specifications applicable to shipment, storage, and handling of the assay assembled and packed by the customer.

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**Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

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