FIREScript® ULTRA H(+) KIT

Catalogue Number	Size (20 µl reactions)
06-53-0000S	20 rxn (sample)
06-53-00050	50 rxn
06-53-00200	200 rxn

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® ULTRA H(+) Reverse Transcriptase (200 U/µl)
10x RT Reaction Buffer with DTT 2.0
RiboGrip® RNase Inhibitor (220 U/µl)

Product description:

- FIREScript® ULTRA H(+) Reverse Transcriptase is an *in silico* engineered reverse transcriptase with full RNase H activity, increased processivity and sensitivity. It is an exceptionally stable enzyme due to a unique patented genetic modification in the polypeptide structure called the Stability TAG**.
- The optimum temperature of the enzyme ranges from **57°C – 62°C**, with the overall working range at 37°C – 65°C. This characteristic is beneficial for the detection of templates with complicated secondary structures.

  NB! Incubation temperatures lower than 50°C need an extended incubation time!
- The enzyme's inherent warm-start capability ensures benchtop stability for up to 24 hours at room temperature and inactivity at lower temperatures leading to less nonspecific amplification.
- FIREScript® ULTRA H(+) Reverse Transcriptase is a fast enzyme with a reaction time of 1–15 minutes and the ability to transcribe over 12 kb RNA sequences. Together with high sensitivity and reproducibility, the enzyme is suitable for routine, high-throughput, and diagnostic applications in RNA workflows.

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

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.
Oligo (dT) primers (100µM)[1] / Random primers (100µM)[1] / Gene specific primer[1]	1 µl	5 µM / 5 µM / 0.1–1 µM[2]
dNTP MIX (20 mM each)[1]	0.5 µl	500 µM
10x RT Reaction Buffer with DTT 2.0	2 µl	1x
FIREScript® ULTRA H(+) Reverse Transcriptase (200 U/µl)[3]	1 µl	10 U/µl
RiboGrip® RNase Inhibitor (220 U/µl)[4]	0.1 µl	1.1 U/µl
Template RNA[1]	0.1–5 µg	Variable
Nuclease-free water[1]	up to 20 µl	
Total	**20 µl**	

[1] Not included in FIREScript® ULTRA H(+) KIT.

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

[3] For one-step RT-qPCR optimization, we recommend a final concentration range of 0.125 – 0.5 U/µl as a starting point. Use the RT enzyme dilution buffer for intermediate dilution.

[4] If there is a need for intermediate dilution, use any 1X RT, RT-(q)PCR, or RT-LAMP reaction buffer to do so.

3. Incubate the reaction mixture as follows:

Step	Temperature	Time
Primer extension (ONLY if using random primers or a mix of random and oligo(dT) primers)	25°C	5–10 min
Reverse transcription	57°C[1]	1–10 min[2]
Enzyme inactivation	85°C	2 min

[1] The optimal working temperature range of the enzyme with regular targets is 57°C – 62°C. Temperatures between 37°C – 56°C and 63°C – 65°C can be applied but require testing of reaction times and efficiency.

[2] Faster reverse transcription times can be used (1-3 minutes), depending on the target and incubation temperature (in the optimal range). For further optimization of the reverse transcription step, refer to the troubleshooting guide.

Priming options:

- Oligo (dT) primers:
  - Specifically bind poly(A) tail of an eucaryotic mRNA (the total RNA pool has 1–2% of poly(A)+ RNA)
  - Generate full-length cDNA
- Random primers:
  - Bind at different sites along an RNA molecule
  - Binding does not depend on the presence of poly(A) tail
- Gene-specific primers:
  - Specific to the gene of interest
  - Increase assay specificity

Considerations while working with RNA:

- Avoid RNase contamination:

    - Wear protective clothing, gloves, *etc.*
    - Use sterile and RNase-free disposables
- Prior to the experiment, store RNA samples at -70°C or below
- To protect RNA template, **always use RiboGrip® RNase Inhibitor** (Cat. no 06-26).
- Thaw and keep RNA samples on ice during the experiment

IMPORTANT:
cDNA synthesis reaction mixture components may inhibit the following 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 37–65°C (for example 37, 42, 50, 55, 60, 65°C) for 10 min. When the optimal temperature is identified the optimal reaction time can be tested (3, 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 - use up to 10% of the cDNA reaction mixture in a final (q)PCR reaction. In case of inhibition, predilute cDNA 5-fold before using in PCR.

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.
- 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 Reverse Transcriptase.

Source:

Purified from an *E. coli* strain that carries an overproducing plasmid containing a sequence coding *FIREScript ULTRA H(+) Reverse Transcriptase*.

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 the 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 set QC stability criteria and is intended to provide guidelines for shipping and storage conditions only. The client or its designee shall be responsible for conducting all necessary stability and functionality 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 client.

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

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Manufacturer: Solis BioDyne OÜ | Teaduspargi 9, 50411 | Tartu, Estonia (EU)