FIREScript® FLEX H(-) Glycerol-Free KIT

Catalogue Number	Size (Units)
06-64-0000S	20 000 U (20 kU) sample
06-64-40000	40 000 U (40 kU)
06-64-0400k	400 000 U (400 kU)

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: 10 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:

Cat No.	Components
06-62	FIREScript® FLEX H(-) Glycerol-Free RT (600 U/µl)
05-77	10x Glycerol-Free RT Reaction Buffer with DTT
06-29	RiboGrip® Glycerol-Free RNase Inhibitor (220 U/µl)

Product description:

- FIREScript® FLEX H(-) **Glycerol-Free** Reverse Transcriptase is an *in silico* engineered reverse transcriptase with reduced RNase H activity, increased processivity and great inhibitor tolerance. It is an exceptionally stable enzyme due to a unique patented genetic modification in the polypeptide structure called the Stability TAG**.
- The glycerol-free formulation supports lyophilization, though, it does not contain lyophilization excipients. The excipients should be added separately based on the final application. The performance is comparable to the glycerol-containing FIREScript® FLEX H(-) Reverse Transcriptase. For detailed guidance on lyophilization, please contact support@solisbiodyne.com.
- The optimum temperature of the enzyme ranges from **58°C – 65°C**, with the overall working range at 37°C – 70°C. This characteristic is beneficial for the detection of templates with complicated secondary structures.

  NB! Incubation temperatures lower than 50°C need extended incubation time!
- The enzyme's inherent warm-start capability keeps it inactive at room temperature and below, allowing reaction setup without ice while reducing nonspecific amplification.

- FIREScript® FLEX H(-) Glycerol-Free Reverse Transcriptase is a fast enzyme with a reaction time of 1–10 minutes and the ability to transcribe over 12 kb RNA sequences. Combined with high processivity, the enzyme delivers reliable performance with low-input, degraded, or challenging RNA samples.
- Wide range of priming options ensures flexible assay design. FIREScript® FLEX H(-) Glycerol-Free 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 thoroughly by gently vortexing or pipetting up and down, followed by brief centrifugation.

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 Glycerol-Free RT Reaction Buffer with DTT[3]	2 µl	1x
FIREScript® FLEX H(-) Glycerol-Free RT (600 U/µl)[4]	0.34 µl	10 U/µl
RiboGrip® Glycerol-Free RNase Inhibitor (220 U/µl)[5]	0.1 µl	1.1 U/µl
Excipient mix[1,6]	Variable	1x
Template RNA[1]	0.5 pg – 5 µg	Variable
Nuclease-free water[1]	up to 20 µl	
Total	**20 µl**	

[1] Not included in FIREScript® FLEX H(-) Glycerol-Free KIT.

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

[3] **NB! Keep 10x Glycerol-Free RT Reaction Buffer with DTT (Cat no. 05-77) out of direct sunlight!**

[4] For one-step RT-qPCR optimization, we recommend a final concentration range of 0.125 – 0.5 U/µl as a starting point. Depending on reaction buffer composition, intermediate dilution of the enzyme to 30 U/µl using 1x Glycerol-Free RT Dilution Buffer (Cat no. 18-22) may improve performance.

[5] For one-step RT-qPCR, we recommend a final concentration around 1 U/µl (optimization as needed).

[6] In case of lyophilization, the excipients (to be selected and supplied by the user) should be added to the mix last. For detailed guidance on lyophilization, please contact support@solisbiodyne.com.

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	60°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 58°C – 65°C. Temperatures between 37°C – 57°C and 66°C – 70°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® Glycerol-Free RNase Inhibitor** (Cat. no 06-29; provided in this kit).
- 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 37–70°C (for example 37, 42, 50, 55, 60, 65, 70°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 to melt stable hairpins/stem-loops, then chill rapidly on ice for 15 min to keep the RNA linear before reverse transcription.

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 – an excess of Reverse Transcriptase relative to the other components can disrupt the reaction balance, reducing efficiency. Reduce the amount of Reverse Transcriptase to restore the optimal ratio.

Source:

Purified from an *E. coli* strain that carries an overproducing plasmid containing a sequence coding *FIREScript FLEX 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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