SolisFAST® 1-step RT-PCR Kit with UNG Ready to Load

Catalogue Number	Size (20 µl reactions)
04-54-0000S	50 reactions (sample)
04-54-00050	50 reactions
04-54-00200	200 reactions
04-54-01000	1 000 reactions

Shipping:

At room temperature

Store at –20°C upon receipt

Batch Number and Expiry Date:

See vial

Storage and Stability*:

- Routine storage at -20°C (–28°C to –18°C) until Expiry Date
- Stable at room temperature (25°C) for 1 month
- Freeze-thaw stability: 10 cycles

Reaction setup:

Recommended setup on ice.

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

Product description:

- SolisFAST® 1-step RT-PCR Kit with UNG Ready to Load provides a simple and streamlined workflow for analyzing RNA targets using reverse transcription PCR. Both the cDNA synthesis and PCR are conveniently performed in one tube within 1 hour, greatly simplifying the analysis workflow, reducing reaction-to-reaction variation and minimizing hands-on steps.
- The product comes in a kit format with 4 tubes and contains all necessary components (except for RNA template, primers) to perform RT-PCR.
- The **4x One-step HOT SolisFAST® Mix with UNG Ready to Load** contains HOT SolisFAST® DNA Polymerase, an *in silico* designed analogue of *Taq* DNA polymerase with chemical hot-start and approximately 2-4 times faster extension rates. The unique buffer system also includes dUTPs and heat-inactivatable Salini UNG® Uracil-N-Glycosylase to prevent carry-over contamination from previous amplifications.
- The kit is premixed with a loading dye (blue and yellow dye with migration equivalents to 3.5-4.5 kb and 35-45 bp DNA fragments, respectively) to enable direct sample loading after the reaction and track DNA during gel electrophoresis.
- The **40x One-step RT Mix** comprises the thermostable SOLIScript® and FIREScript® Reverse Transcriptases active at temperatures up to 60°C, beneficial when using templates with high levels of secondary structures. It also includes the RNase Inhibitor RiboGrip® that inactivates commonly encountered RNases (A, B and C) and prevents RNA degradation.

Content:

Component	Product sizes and Volumes			
	Sample (50 rxn)	50 rxn	200 rxn	1000 rxn
4x One-step HOT SolisFAST® Mix with UNG Ready to load	250 µl	250 µl	1 ml	5 ml
40x One-step RT Mix	25 µl	25 µl	100 µl	500 µl
5x AmpliBoost™ RT-PCR Enhancer	200 µl	200 µl	800 µl	4 ml
Water, nuclease-free	1.25 ml	1.25 ml	5 ml	4x 5 ml

Note: To avoid repeated freeze-thaw cycles as well as to minimize the contamination risk of stock solutions of reagents, it is highly recommended to divide large-volume stocks into several smaller aliquots and store them at -20°C.

Mix compositions:

Kit component	Description
4x One-step HOT SolisFAST® Mix with UNG Ready to Load	HOT SolisFAST® DNA polymerase, Salini UNG® Uracil-N-Glycosylase, optimized reaction buffer, dNTPs including dUTP, 6.4 mM $MgCl_2$ (1x qPCR solution – 1.6 mM $MgCl_2$), mix of blue and yellow loading dyes.
40x One-step RT Mix	FIREScript® and SOLIScript® Reverse Transcriptases, RiboGrip® RNase Inhibitor.
5x AmpliBoost™ RT-PCR Enhancer	A novel RT-PCR enhancer to improve sensitivity and performance with GC-rich and other challenging amplicons.

Step-by-step guidelines:

1. Thaw the reagents. Mix each reagent by gentle vortexing or pipetting up and down, then centrifuge briefly.

2. Prepare a reaction mix on ice. Add all components except for template RNA. **NOTE**: Include no-template (NTC) control. In NTC replace RNA with the same amount of nuclease-free water.

Component	Volume [a]	Final conc.
4x One-step HOT SolisFAST® Mix with UNG Ready to Load	5 µl	1x
40x One-step RT Mix	0.5 µl	1x
Forward Primer (10 µM)	0.5 µl	250 nM
Reverse Primer (10 µM)	0.5 µl	250 nM
5x AmpliBoost™ RT-PCR Enhancer (optional) [b]	4 µl [b]	1x [b]
Template RNA	Variable [c]	0.1 pg–1 µg (total RNA)
Water, nuclease-free	up to 20 µl	
Total reaction volume	**20 µl**	

[a] Scale all components proportionally according to sample number and reaction volumes. Make sure you use enough of each reagent for your reactions, plus 10% extra volume to accommodate pipetting errors. For high reaction efficiency, do not exceed 20 µl reaction volumes.

[b] Include 5x AmpliBoost™ RT-PCR Enhancer for improved sensitivity and yield with low-concentratedsamples, GC-rich or other challenging templates. The final concentration can be optimized from 0.5x – 1.5x. Note: Due to the buffer composition, use of AmpliBoost™ can lead to smeared bands in gel electrophoresis.

[c] See pages 6–7 for recommendations on primers design, template preparation and additional guidelines.

3. Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of reaction mix into PCR wells.

4. Add template RNA to the PCR wells. Seal the wells using the procedure recommended for the cycling instrument being used and centrifuge the reactions briefly.

5. Recommended cycling conditions are shown in the table below. The program can be optimized depending on assay design (see 5[a,b,c,d]).

Cycling protocol for singleplex reactions

		Temperature	Time
UNG treatment [a]		25°C	30 sec
Reverse transcription [b]		50°C	5 min
Enzyme activation [c]		95°C	10 min
35 cycles	Denaturation [d]	95°C	5 sec
	Annealing/Extension [d]	55-65°C	5 sec
	Extension	72°C	8 sec
Final extension		72°C	1 min

[a] Use UNG treatment step if your experimental set-up needs UNG functionality (to remove carryover contamination). UNG is fully active between 25°C to 40°C and inactive at reaction temperatures above 50°C. This step can be skipped if UNG treatment is not used.

[b] A 50°C RT step temperature is optimal for SOLIScript® and FIREScript® Reverse Transcriptases. Temperatures from 45 to 60°C can be tested for optimization. For difficult templates with a high level of secondary structures, the temperature of reverse transcription may be increased to 60°C.

[c] IMPORTANT: Enzyme activation step at **95°C for 10 min** is crucial for full activation of DNA polymerase.

[d] The cycling program can be optimized depending on the instrument specifications and assay design. Annealing temperature is dependent on the melting temperature of the primers. Performing a gradient PCR to determine the most optimal annealing temperature is recommended.

Cycling protocol for multiplex reactions [e]

		Temperature	Time
UNG treatment [a]		25°C	30 sec
Reverse transcription [b]		50°C	15 min
Enzyme activation [c]		95°C	10 min
40 cycles	Denaturation [d]	95°C	5 sec
	Annealing/Extension [d]	55-65°C	30 sec
	Extension	72°C	30 sec
Final extension		72°C	4 min

[e] See the previous table for footnotes.

IMPORTANT: To avoid degradation of PCR products by residual activity of UNG enzyme, amplification products should be stored at –20°C. Storage at room temperature for 24 hours has no detrimental effect on PCR products.

Recommendations for a successful PCR experiment

Prerequisites for a successful PCR experiment include the design of optimal primer pairs, the use of high-quality template RNA and appropriate concentrations of reaction components. Below are a few recommendations that may help to achieve the best results.

Primers

1. Use dedicated software, such as Primer3 (bioinfo.ut.ee/primer3) or NCBI Primer-BLAST (ncbi.nlm.nih.gov/tools/primer-blast) to design target-specific primers.
2. The optimal primer length is 18–24 bp, with GC-content 35–65% and calculated melting temperatures (Tm) 60–70°C. Tm of the two primers should not differ by more than 3°C.

3. Analyze your primers for self-complementarity and stable secondary structures. Avoid the 3'-self complementarity, because it increases probability of primer-dimers formation.
4. In case of multiplexing, check the primers for cross-reactivity and minimize Tm mismatches. Test the efficiency of your PCR experiment in individual assays before combining them in a multiplex assay.

Template

1. The integrity, purity and concentration of the RNA template should be suitable for the PCR experiment. Ratios of the absorbance at 230, 260, and 280 nm are used to assess the purity of nucleic acid samples. High quality sample has 260/280 and 260/230 ratios equal or higher than 1.8 and 2.0, respectively. The presence of contaminants such as phenol, carbohydrates or EDTA may compromise the results.
2. Treat your purified RNA sample with RNase-free DNase I to remove contaminating genomic DNA which can act as a template in PCR reaction or design primers that prevent gDNA amplification.
3. To monitor possible contamination and primer-dimer formation always include a no-template control (NTC) by replacing the DNA template with the same volume of nuclease-free water.

Troubleshooting

Please refer to our Troubleshooting Guide or more information.

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

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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.

This product is supplied for research use only. It is suitable for use as a component of molecular diagnostic assays, where applicable country laws allow. This product alone does not provide any diagnostic result. Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.