SOLIScript® Fast 1-step RT-qPCR Mix with UNG

Catalogue Number	Size (20 µl reactions)
08-87-0000S	50 reactions (sample)
08-87-00200	200 reactions
08-87-00200-5	5 x 200 reactions
08-87-05000	5 000 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 4°C (2°C to 8°C) for 3 months
- Stable at room temperature (25°C) for 1 month
- Freeze-thaw stability: 20 cycles**

**The Mix is normally liquid at –20°C. Occasional temperature fluctuations may cause the product to freeze solid. This does not affect the functionality of the Mix.

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:

- SOLIScript® Fast 1-step RT-qPCR Mix with UNG is a 4x concentrated mix optimized for sensitive and accurate quantification of RNA targets by real-time one-step RT-PCR (RT-qPCR) using dual-labeled hydrolysis probes (e.g., TaqMan® probes), and is suitable for detection and quantitation of up to five targets simultaneously. Inhibitor tolerance and fast cycling compatibility enable flexible experiment design.
- The Mix comes in 1 tube (nuclease-free water supplied in a separate tube) and contains all necessary components (except for RNA template, primers and probe(s)) to perform RT-qPCR.
- The Mix comprises the *in silico* engineered thermostable SOLIScript® Reverse Transcriptase 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 RNase A, RNase B and RNase C (III) to prevent RNA degradation and increase cDNA yield in reactions with low RNA amounts.
- The Mix 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-inactivated Salini UNG™ Uracil-N-Glycosylase to prevent carry-over contamination from previous amplifications. UNG is fully active between 25°C to 40°C and inactive at reaction temperatures above 50°C.

Reagents supplied:

	Product sizes and Volumes			
Component	**50 rxn**	**200 rxn**	**5x200 rxn**	**5000 rxn**
4x SOLIScript® Fast 1-step RT-qPCR Mix with UNG	250 µl	1 ml	5 x 1 ml	25 ml
Water, nuclease-free	1.25 ml	5 ml	3 x 5 ml	100 ml

Note: To avoid repeated freezing and thawing 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 composition:

SOLIScript® Fast 1-step RT-qPCR Mix with UNG comprises:

- SOLIScript® Reverse Transcriptase
- RiboGrip™ RNase Inhibitor
- HOT SolisFAST® DNA polymerase
- Salini UNG™ Uracil-N-Glycosylase
- A unique reaction buffer with dNTPs (incl. dUTPs), 22.0 mM $MgCl_2$ (1x RT-qPCR solution – 5.5 mM $MgCl_2$) and additives to maximize PCR efficiency

Compatible real-time instruments:

The product is compatible with most qPCR cyclers. If using a ROX-dependent platform, switch off ROX normalization prior to the run. Version with ROX is available upon request as a custom product (contact support@solisbiodyne.com).

Step-by-step guidelines:

1. Place the reagents on ice. Mix each reagent by gentle vortexing or pipetting up and down, then centrifuge briefly. **NOTE**: Minimize the exposure of the fluorescently labelled probe(s) to light.
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 SOLIScript® Fast 1-step RT-qPCR Mix with UNG	5 µl	1x
Forward Primer (10 µM)	1.2 µl	0.6 µM [b]
Reverse Primer (10 µM)	1.2 µl	0.6 µM [b]
Probe (10 µM)	0.5 µl	0.25 µM [b]
Template RNA	Variable [b]	1 pg–1 µg (total RNA)
Water, nuclease-free	up to 20 µl	
Total reaction volume	**20 µl**	

[a] Reaction volume can be scaled down to 10 µl. Scale components proportionally according to sample number and reaction volumes. Use enough of each reagent for your number of reactions and add 5–10% extra volume to accommodate pipetting errors.

[b] Optimal results may require titration of primer and probe concentrations. A final concentration of 0.3-0.9 µM of each primer and 0.1–0.3 µM of each probe is suitable for most applications. Volume of template RNA depends on template concentration and assay design. See pages 6–7 for Recommendations on primers/probe 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]). **NOTE**: When programming your RT-qPCR run, choose the detection channel(s) of the qPCR instrument that correspond to the fluorophore label(s) of the target-specific probe(s) present in the assay. Acquisition of real-time data generated by fluorogenic probe(s) should be performed as recommended by the instrument's manufacturer.

		Temperature	Time
UNG treatment [a]		25°C	30 sec
Reverse transcription [b]		50°C	5 min
Enzyme activation [c]		95°C	10 min
45 cycles	Denaturation [d]	95°C	3 sec
	Annealing/Extension [d]	60°C	20 sec

[a] Use UNG treatment step if your experimental set-up needs UNG functionality (to remove carryover contamination).

[b] A 50°C RT step temperature is optimal for SOLIScript® Reverse Transcriptase. 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. Denaturation time of 1–5 sec and annealing/extension time of 5–20 sec is recommended. Annealing/extension temperature is dependent on the melting temperature of the primers and DNA probe used. Performing a gradient PCR to determine the most optimal annealing/extension temperature is recommended.

6. Collect and analyze the data according to the instrument-specific instructions. Check replicates and amplification plot profile. Set up a standard curve if absolute quantification is desired.

Recommendations for a successful RT-qPCR experiment

Template:

1. 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. Input material can range from 1 pg to 1 µg of purified total RNA per 20 µl reaction. To determine the optimal template amount (especially when amplifying more that one target and/or using other types of input RNA, e.g. mRNA or viral RNA), perform your reactions on a serially diluted template (e.g., 10-fold dilution series). Select the quantity that produces the earliest Ct without inhibiting your amplification.

Primers:

1. Use dedicated software, such as open source Primer3 and NCBI Primer-BLAST to design target-specific primers. To prevent genomic DNA amplification, design primers spanning exon-exon junctions of the target mRNA, or primers that hybridize with sequences in consecutive exons separated by a long (e.g., 1 kb)

intron. Amplification of more than one target in a single reaction may require considerable optimization, including the validation of individual assays prior to multiplexing.

2. Suitable primers are typically 18–24 bp long, with GC-content of 35–65 %, targeting a 50–150 bp amplicon. The optimal melting temperature (T_m) of the primers is 58–60°C. Primers with T_m outside of this range and/or amplicons longer than 150 bp may require optimization of cycling conditions. For multiplex reactions, design primer pairs with similar annealing temperatures for all targets to be amplified. Self- and cross-complementarity of primers and probes should be avoided.

3. Optimal results may require titration of primer concentration between 0.3 and 0.9 µM. A final concentration of 0.6 µM for each primer is suitable for most applications. In multiplex reactions, different targets may require different primer concentrations.

Probe:

1. Fluorescent reporter dye(s) and quencher(s) in RT-qPCR analysis should be chosen according to the detection capabilities of the qPCR platform used. Refer to your instrument manufacturer's guidelines for information specific to your particular instrument.

2. The optimal length of the probe is 9–40 bp (hydrolysis probes: up to 30 bp). The probe binding site should be in close proximity to the forward or reverse primer (4–15 bp distance between 3' end of primer and 5' end of probe is recommended). The binding sites of primers and probes should not overlap. Probe can bind to either strand of the target.

3. Tm of the probe should be 6–8°C higher than the Tm of the primers. In case of a suboptimal probe Tm, less of the probe is

bound to the target sequence. This may result in compromised sensitivity, reduced fluorescence signal and inadequate representation of the target amount in the sample.

4. Optimal results may require titration of probe concentration between 0.1 µM and 0.4 µM. A final probe concentration of 250 µM is suitable for most applications. In multiplex reactions, different targets may require different probe concentrations.

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

DS-08-87 v2
Effective from: 20.03.2023

* 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.

SOLIS BIODYNE