SOLIS BIODYNE

SOLIScript® 1-step Probe Kit

Catalogue Number	Pack Size
08-57-0000S	50 x 20 µl reactions
08-57-00250	250 x 20 µl reactions

Shipping:
At room temperature

Batch Number and Expiry Date:
See vial

Stability at room temperature 1 MONTH

Store at –20°C

Storage and Stability:
- Routine storage at - 20°C until expiry date
- Can be stored at +4°C for up to 6 months
- Stability at room temperature (15–25°C) for 1 month
- Freeze-thaw stability: 30 cycles
- Store 5x One-step Probe Mix protected from light

Reaction setup:
At room temperature

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

Product description:
- SOLIScript® 1-step Probe Kit is optimized for highly 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). The Kit is suitable for detection of up to two targets simultaneously. This is the optimal solution for gene expression studies where an endogenous control assay is detected in the same well as the target of interest.
- The Kit comes in 3 tubes (including water) and contains all the components necessary (except RNA template, primers, probe) to perform RT-qPCR in a single tube, and is compatible with ROX-dependent and ROX-independent qPCR cyclers.
- 40x One-step SOLIScript® Mix comprises in silico-engineered SOLIScript® Reverse Transcriptase and RiboGrip™ RNase Inhibitor. SOLIScript® is a thermostable reverse transcriptase active at temperatures up to 60°C, beneficial when using templates with high levels of secondary structure. RiboGrip™ inactivates RNase A to protect RNA sample from degradation and increase cDNA yield in reactions with low RNA amounts.
- 5x One-step Probe Mix contains HOT FIREPol® DNA Polymerase in a unique reaction buffer maximizing the performance of both reverse transcriptase and DNA polymerase in a single assay, and minimizing the formation of primer-dimers and other non-specific PCR amplifications.
- The Kit can be used for gene expression analysis and low-copy gene detection, gene knockdown validation, miRNA profiling and quantification, characterization of GMOs, RNA viral pathogen detection and quantification.

Content:

Component	Catalogue Number 08-57-0000S 50 x 20 µl rxn	Catalogue Number 08-57-00250 250 x 20 µl rxn
40x One-step SOLIScript® Mix	25 µl	125 µl
5x One-step Probe Mix	200 µl	1000 µl
Water, nuclease-free	1.25 ml	5.0 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. Store 5x One-step Probe Mix protected from light.

Mix compositions:

Kit component	Description
40x One-step SOLIScript® Mix	SOLIScript® Reverse Transcriptase, RiboGrip™ RNase Inhibitor
5x One-step Probe Mix	HOT FIREPol® DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), 15 mM $MgCl_2$ (1x RT-qPCR solution – 3 mM $MgCl_2$), passive reference dye based on ROX dye

Compatible real-time instruments:

The Kit is compatible with ROX-independent and ROX-dependent qPCR platforms.

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. **NOTE**: Minimize the exposure of the 5x One-step Probe Mix and fluorescently labelled probe(s) to light.
2. Prepare a reaction mix. Add all components except the template RNA. **NOTE**: Include a no-template (NTC) and no-RT control (NRT). In NTC replace RNA, and in NRT replace 40x One-step SOLIScript® Mix with corresponding amounts of nuclease-free water. If combining different assays on a single plate, include at least one NRT for each assay for every RNA.

Component	Volume[1]	Final conc.
40x One-step SOLIScript® Mix	0.5 µl	1x
5x One-step Probe Mix	4 µl	1x
Forward Primer (10 µM)[2]	0.8 µl	400 nM
Reverse Primer (10 µM)[2]	0.8 µl	400 nM
Probe[2]	Variable	250 nM
Template RNA[2]	Variable	1 pg–1 µg (total RNA)
Water, nuclease-free	up to 20 µl	
Total reaction volume	**20 µl**	

[1] Reaction volume can be scaled from 10 to 50 µl. Scale components proportionally according to sample number and reaction volumes. Use enough of each reagent for your reactions, plus 5–10% extra volume to accommodate pipetting errors.

[2] See page 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 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. Run your RT-qPCR assay as shown in table below. **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
Reverse transcription[1]		50°C	15 min
Enzyme activation[2]		95°C	10 min
40 cycles	Denaturation	95°C	15 sec
	Annealing/Extension	60°C	60 sec

[1] A 50°C RT step temperature is optimal for SOLIScript® Reverse Transcriptase. SOLIScript® Reverse Transcriptase is active at 45–60°C. For difficult templates with high secondary structure, the temperature may be increased to 60°C.

[2] IMPORTANT: Enzyme activation step at 95°C for 10 min is crucial for full activation of DNA polymerase and inactivation of reverse transcriptase.

6. Collect and analyze the data according to the instrument-specific instructions. Verify the amplification curve, 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. "Good RNA" 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 template during reaction.

3. Starting material can range from 1 pg to 1 g of purified total RNA per 20 µl reaction. To determine the optimal template quantity (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 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. Amplification of more than one target in a single reaction may require considerable optimization, individual assays must be validated prior to multiplexing.

2. Suitable primers are typically 18–24 bp long, with GC-content 35–65 %, and target 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-complementarity of primers should be avoided.

3. Optimal results may require titration of primer concentration between 200 and 600 nM. A final concentration of 400 nM each primer is suitable for most applications.

Probe:

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

2. The length of the probe is 9–40 bases (hydrolysis probes: up to 30 bases). The probe binding site should be in close proximity to the forward or reverse primer; however, binding sites 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 the Tm of the probe is not optimal, less of the probe is bound to the target sequence. This may result in compromised sensitivity, reduced fluorescence signal and not a true representation of the target amount in the sample.

4. Optimal results may require titration of probe concentration between 100 and 250 nM. A final concentration of each 250 nM probe is suitable for most applications.

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-08-57 v2

Revised 08.06.2020

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