SOLIScript® 1-step SolisGreen® Kit 2.0

Catalog Number	Size (20 µl reactions)
08-91-0000S	50 reactions (sample)
08-91-00250	250 reactions

Store at –20°C upon receipt

Shipping:

At room temperature.

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 up to 3 months
- Stable at room temperature (25°C) for up to 1 month
- Freeze-thaw stability: 15 cycles
- Store 5x One-step SolisGreen® Mix 2.0 protected from light

Reaction setup:

At room temperature

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

Product description:

- **SOLIScript® 1-step SolisGreen® Kit 2.0** is optimized for highly sensitive and accurate quantification of RNA targets by real-time one-step RT-PCR (RT-qPCR) using dsDNA-intercalating dye detection via the SYBR®/FAM fluorescence channel.
- Compatible with ROX-independent and ROX-dependent qPCR cyclers (excluding high-ROX platforms).
- The Kit comes in 3 tubes (including water) and contains all the components necessary (except RNA template and primers) to perform cDNA synthesis and qPCR in a single tube.
- **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 SolisGreen® Mix 2.0** contains HOT FIREPol® DNA Polymerase, SolisGreen®** dsDNA intercalating dye and a ROX-based passive reference dye 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.

Content:

Component	Catalog Number	
	08-91-0000S 50 x 20 µl rxn	08-91-00250 250 x 20 µl rxn
40x One-step SOLIScript® Mix	25 µl	125 µl
5x One-step SolisGreen® Mix 2.0	200 µl	1000 µl
Nuclease-free water	1.25 ml	5.0 ml

Kit components:

Kit component	Description
40x One-step SOLIScript® Mix	SOLIScript® Reverse Transcriptase, RiboGrip® RNase Inhibitor
5x One-step SolisGreen® Mix 2.0	HOT FIREPol® DNA polymerase, dNTPs (dATP, dCTP, dGTP, dTTP), 12.5 mM $MgCl_2$ (1x RT-qPCR solution – 2.5 mM $MgCl_2$), SolisGreen® dsDNA-intercalating dye, passive reference dye based on ROX dye

Applications:

Recommended for various RNA applications, such as:

- Gene expression analysis and low-copy gene detection
- Gene knockdown validation
- miRNA profiling and quantification
- Characterization of GMOs
- RNA viral pathogen detection and quantification

Compatible real-time instruments:

The Kit is compatible with ROX-independent and low-ROX qPCR cyclers where ROX is used as a passive reference signal for normalization of the data (please see the compatibility info on page 7).

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**: Protect 5x One-step SolisGreen® Mix 2.0 from light during thawing and reaction set-up.

2. Prepare a reaction mix at room temperature. Add all required components except the template RNA. Include a no-template control (NTC) and no-RT control (NRT). In NTC replace RNA sample, and in NRT replace 40x One-step SOLIScript® Mix with corresponding amounts of nuclease-free water. **Note**: Include at least one NRT for every RNA sample used in the assay.

Component	Volume[a]	Final conc.
40x One-step SOLIScript® Mix	0.5 µl	1x
5x One-step SolisGreen® Mix 2.0	4 µl	1x
Forward Primer (10 µM)[b]	0.8 µl	400 nM
Reverse Primer (10 µM)[b]	0.8 µl	400 nM
Template RNA[b]	Variable	1 pg–1 µg (total RNA)
Nuclease-free water	up to 20 µl	
Total reaction volume	**20 µl[a]**	

[a] Final reaction volume can be scaled from 10 to 50 µl depending on the qPCR platform and reaction plate (e.g. 96-well or 384-well plates). For multiple reactions, preparation of a master mix of common components is crucial to reduce pipetting

errors. 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.

[b] See page 6-7 for recommendations on primer 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. Program the thermal cycler using the cycling conditions recommended below. Optimal cycling conditions may vary depending on primers. In most cases, a 2-step protocol is recommended. A 3-step protocol may improve assay performance with some primer sets and amplicons longer than 150 bp.

		2-step protocol	3-step protocol
Reverse transcription[a]		50 °C, 15 min	50 °C, 15 min
Enzyme activation[b]		95 °C, 10 min	95 °C, 10 min
40 cycles	**Denaturation**	95 °C, 15 sec	95 °C, 10 sec
	Annealing	60 °C, 1 min	60 °C, 20 sec
	Extension		72 °C, 20 sec
Melt curve analysis[c]		follow instrument recommendations	

[a] RT step at 50 °C is optimal for SOLIScript® Reverse Transcriptase, however the reaction can be performed at temperatures 45–60 °C. For difficult templates with high secondary structure, the temperature may be increased to 60 °C.

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

6. Place the reactions into the qPCR cycler, and start the qPCR run.

7. Collect and analyze the data according to instrument-specific instructions. Verify the amplification curve and melting curve, set up a standard curve if absolute quantification is desired.

Recommendations for a successful 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 of the experiment.
2. Starting material can range from 1 pg to 1 µg of purified total RNA per 20 µl reaction. To determine the optimal template quantity, perform your reactions on a serially diluted template (e.g. 10-fold dilution series). Select the quantity that results in the earliest Ct without inhibiting the amplification.
3. Treat your purified RNA with RNase-free DNase I to remove contaminating gDNA which can act as a template during reaction. Include a no-template (NTC) and no-RT (NRT) control. The NTC will enable detection of contamination of reaction components. The NRT will enable detection of contaminating gDNA. If combining assays with different primer pairs on a single plate, include at least one NRT for each primer pair for every RNA sample used in the assay.

Primers

1. Use dedicated software, such as open source Primer3 and NCBI Primer-BLAST to design target-specific primers. In order 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.

2. Suitable primers are typically 18–24 bp long, with GC-content 35–65%, and target a 50–150 bp amplicon. Runs of more than four consecutive G or C bases should be avoided. Targets longer than 150 bp may require a 3-step cycling protocol (page 5). The optimal melting temperature (Tm) of the primers is 58–60 °C. Primers with T_m outside of this range may require optimization of cycling conditions. Analyze your PCR primers for self-complementarity. Avoid 3'-self complementarity, because it increases the possibility of primer-dimers formation.
3. A final primer concentration of 400 nM (of each) is suitable for most PCR conditions. If your primers do not amplify efficiently, determine an optimal primer concentration using primer titration (100–600 nM). In some cases, higher concentration of the reverse primer alone may improve RT-qPCR efficiency without compromising amplification specificity. Always include a post-amplification melt curve analysis to verify the specificity of amplification and to check for the presence of non-specific amplification products.

Cycling

SOLIScript® 1-step SolisGreen® Kit 2.0 is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM.

SOLIScript® 1-step SolisGreen® Kit 2.0 is not compatible with qPCR cyclers that require high ROX levels for signal normalization, such as Applied BioSystems® 7900HT, StepOne™ or StepOnePlus™ systems.

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

DS-08-91 v1
Effective from: 20.05.2024

*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.

Permitted Use: This product is supplied for research use only (the Permitted Use). Some applications of this product may require a license/licenses from Solis BioDyne OÜ or one or more third parties which are not provided by the purchase of this product. This product shall comply with its relevant specification and be fit for its stated purpose, Solis BioDyne OÜ gives no other warranty and makes no representation as to description or quality. For more information and full disclaimers, please contact our customer service.

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

SOLIS BIODYNE