SolisFAST® SolisGreen® qPCR Mix (ROX), 5X

Catalogue Number	Pack Size	20 µl rxn
28-46-0000S	0.2 ml	50
28-46-00001	1 ml	250
28-46-00001-5	5 x 1 ml	5 x 250
28-46-00020	20 ml	5000

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 12 months
- Stable at room temperature (25°C) for 3 months
- Freeze-thaw stability: 30 cycles

Reaction setup:
At room temperature

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

Product description:

- SolisFAST® SolisGreen® qPCR Mix (ROX) is a 5X-concentrated, ready-to-use solution optimized for fast, highly sensitive and reproducible dye-based qPCR assays.
- The mix contains all components necessary, except primers, nuclease-free water, and DNA template.

Mix component	Description
SolisFAST® DNA Polymerase	*In silico* designed analogue of *Taq* DNA polymerase with enhanced stability at room temperature due to a genetic modification – Stability TAG**, fast hot-start and faster extension rates compared to the wild-type *Taq DNA* polymerase
qPCR buffer	Includes 7.5 mM $MgCl_2$ (1X PCR solution 1.5 mM $MgCl_2$), dNTPs (dATP, dCTP, dGTP, dTTP), stabilizers and enhancers that maximize efficiency of PCR reaction
Fluorescent dye	SolisGreen® is a nucleic acid dye detected in the FAM or SYBR® Green I channel
Reference dye	ROX is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR

Compatible real-time instruments:

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

Step-by-step guidelines:

1. Thaw SolisFAST® SolisGreen® qPCR Mix, template DNA, primers, and nuclease-free water. Mix each component by gentle vortexing or pipetting up and down, then centrifuge briefly.
2. Prepare a reaction mix. Add all required components except the template DNA.

Component	Volume[a]	Final conc.
SolisFAST® SolisGreen® qPCR Mix (ROX) (5X)	4 µl	1X
Forward Primer (10 µM)	0.6 µl	300 nM
Reverse Primer (10 µM)	0.6 µl	300 nM
Template DNA (added at step 4)	Variable	cDNA: < 100 ng gDNA: < 50 ng
Nuclease-free water	up to 20 µl	
Total reaction volume	**20 µl[a]**	

[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.

3. Mix the reaction mix thoroughly, then centrifuge briefly. Dispense appropriate volumes of mix into PCR wells.
4. Add template DNA to the PCR wells.
5. Seal the wells using the procedure recommended for the cycling instrument being used, and centrifuge the reactions briefly.

6. Program the thermal cycler using the cycling conditions recommended below.

Step	Temperature	Time	Cycles
Enzyme activation[a]	95°C	30 sec–3 min[a]	1
Denaturation	95°C	5 sec[b]	40
Annealing/extension	57°C[c]	20 sec[b]	
Melt curve analysis[d]	60–95°C	various	1

[a] 30 sec is enough for enzyme activation; for complex templates (gDNA) 2 min is recommended to fully denature DNA

[b] The cycling program can be optimized depending on the instrument specifications, assay design and speed requirements. Denaturation time of 1–5 sec and annealing/extension time of 5–20 sec is recommended. 20 sec of annealing/extension is suitable for all qPCR cyclers listed on page 7.

[c] Annealing/extension temperature is dependent on the melting temperature (Tm) of the primers used. Generally, the most optimal annealing/extension temperature is 2-5 °C below the Tm. Performing gradient PCR to determine the most optimal annealing/extension temperature is recommended.

[d] Follow real-time instrument recommendations for melt curve analysis

7. Place the reactions into the qPCR cycler, and start the qPCR run.
8. After the reaction is completed, perform data analysis according to the instrument-specific instructions.

Recommendations for a successful qPCR experiment

Primers

Use primer-design software, such as Primer3 (bioinfo.ut.ee/primer3) or NCBI Primer-BLAST (ncbi.nlm.nih.gov/tools/primer-blast) to design target-specific primers.

1. For best qPCR efficiency design primers targeting an amplicon size of 50 to 150 bp.
2. The optimal length of primers is 20–30 bases.
3. Avoid runs of more than four consecutive G or C bases.
4. GC-content should range from 40–60%. Higher GC-content may need more optimization by adding DMSO, betaine or other additives to improve the results.
5. The optimal melting temperature (T_m) of the primers is ~60-63°C. In order for both primers to bind efficiently, the T_m of the two primers should not differ by more than 3°C. Generally, the most optimal annealing/extension temperature is 2-5 °C below the Tm (annealing/extension temperature of 57-60°C is suitable in most cases). Performing gradient PCR to determine the most optimal annealing/extension temperature is recommended.
6. Analyze your PCR primers for self-complementarity in their sequences. Avoid 3'-self complementarity, because it increases the possibility of primer-dimers formation.
7. A final primer concentration of 300 nM is suitable for most PCR conditions. If your primers do not amplify efficiently, determine an optimal primer concentration using primer titration in the range of 0.2–0.5 µM.

Template

The integrity, purity and concentration of the DNA template should be suitable for the qPCR experiment.

1. The template needs to be purified of PCR inhibitors (e.g. EDTA).
2. The recommended final concentration of DNA template for the qPCR experiment is dependent upon the type of DNA used. For example, if you use cDNA as a template, qPCR efficiency would be largely dependent on the expression level of the target gene. We recommend using up to 50 ng of complex (e.g. eukaryotic) DNA and up to 100 ng of cDNA in your reactions.
3. The recommended final amount of cDNA sample in the qPCR reaction mixture is up to one tenth of the final qPCR reaction volume. Overload of cDNA sample may compromise the reaction, because the cDNA sample may contain reaction components inhibiting your qPCR.
4. Perform and analyze your qPCR reactions in triplicates on a serially diluted template (e.g. 10-fold dilution series).
5. Dilutions should be done in deionized water and should be prepared fresh before each experiment. Use the standard curve derived from the serial dilutions to assess qPCR efficiency and to determine the optimal template concentration for your qPCR assay.
6. To monitor possible contamination and primer-dimer formation, always include a no-template control (NTC), replacing the DNA template with nuclease-free water.

Cycling

The cycling conditions are optimized for assays with a primer T_m of 60°C, and are compatible with the qPCR instruments listed in the following table:

Manufacturer	Model
Applied Biosystems	7500, 7500 Fast, 7900HT Fast
	QuantStudio™ 3, 5, 6 Flex, 7 Flex, 12K Flex
	ViiA™ 7
Agilent Technologies	Mx3000P™, Mx3005P™, Mx4000™

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

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Reason for revision: product stability info updated page 1 and 8. Stability TAG info added page 2. Trademark and Permitted Use info updated page 8. Technical experiment recommendations renewed.

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