HOT FIREPol® SolisGreen® qPCR Mix 2.0

Catalog Number	Size (20 µl reactions)
08-47-0000S	50 reactions (sample)
08-47-00001	250 reactions
08-47-00001-5	1250 reactions
08-47-00001-10	2500 reactions
08-47-00020	5 000 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 the product 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:

- **HOT FIREPol® SolisGreen® qPCR Mix 2.0** is a 5x-concentrated ready-to-use solution for highly sensitive and reproducible dye-based qPCR assays.
- **HOT FIREPol® SolisGreen® qPCR Mix 2.0** comprises all the components necessary, excluding the template and primers, to perform highly sensitive qPCR. The user simply needs to add water, template, and primers.

Mix component	Description
HOT FIREPol® DNA Polymerase	A chemically modified FIREPol® DNA Polymerase which is activated by a 10 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.
5x qPCR buffer	Includes 12.5 mM $MgCl_2$ (1X PCR solution 2.5 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.

Applications:

Recommended for various DNA applications, such as:

- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination

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 info on page 6).

Step-by-step guidelines:

1. Thaw HOT FIREPol® SolisGreen® qPCR Mix 2.0, 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.
HOT FIREPol® SolisGreen® qPCR Mix 2.0 (5X)	4 µl	1X
Forward Primer (10 µM)	0.16–0.5 µl	80–250 nM
Reverse Primer (10 µM)	0.16–0.5 µl	80–250 nM
Template DNA (added at step 4)	Variable	cDNA: 0.002–200 ng gDNA: 0.2–80 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.

[b] See pages 5-6 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 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	10 min[a]	1
Denaturation	95°C	10 sec	40
Annealing	60°–65°C[b]	20 sec	
Extension	72°C	20 sec	
Melt curve analysis[c]	60–95°C	various	1

[a] To activate the polymerase, include an incubation step at 95°C for 10 minutes at the beginning of the qPCR cycle.

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

[c] 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 T_m (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 250 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 80–250 nM.

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 80 ng of complex (e.g. eukaryotic) DNA and up to 200 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

HOT FIREPol® SolisGreen® qPCR Mix 2.0 is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM.

HOT FIREPol® SolisGreen® qPCR Mix 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-08-47 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.

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