HOT FIREPol® Probe Universal qPCR Kit

Catalogue Number	Pack Size	20 µl rxn
08-88-0000S	0.2 ml	50
08-88-00250	1 ml	250
08-88-00250-5	5 x 1 ml	5 x 250
08-88-05000	20 ml	5000

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 until expiry date.
- The mix 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.

Reaction setup:

At room temperature.

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

Product description:

- HOT FIREPol® Probe Universal qPCR Kit is an optimized ready-to-use solution for highly sensitive and reproducible probe-based real-time quantitative PCR assays.
- The kit contains all components necessary to perform single- or duplex qPCR, except primers, nuclease-free water, and DNA template.
- The kit is optimized for dual-labeled hydrolysis probes (e.g.TaqMan® probes) based on the 5' exonuclease activity.
- Efficient amplification of regular GC- and AT-rich targets. 100% DMSO is included in the kit in a separate vial. DMSO is recommended as a PCR additive for templates with high GC content or strong secondary structures. The most optimal final concentration is between 2.5% and 10%.

Kit content:

Component	Catalogue Number			
	08-88-0000S	08-88-00250	08-88-00250-5	08-88-05000
HOT FIREPol® Probe Universal qPCR Mix, 5x	0.2 ml	1 ml	5 x 1 ml	20 ml
100% DMSO	0.2 ml	0.5 ml	5 x 0.5 ml	10 ml

5x HOT FIREPol® Probe Universal qPCR Mix components:

Mix component	Description
HOT FIREPol® DNA Polymerase	A chemically modified hot-start version of the thermostable Taq DNA polymerase FIREPol® that possesses enhanced stability at room temperature.
qPCR buffer	Includes 15 mM $MgCl_2$ (1X PCR solution – 3 mM $MgCl_2$) and dNTPs (including dUTPs)
ROX dye	Internal reference dye. The mix is suitable for both ROX-dependent and ROX-independent qPCR cyclers.[1]

[1] If ROX dye is used as a reporter fluorophore, internal reference might interfere with signal detection.

Step-by-step guidelines:

1. Thaw HOT FIREPol® Probe Universal 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	10 µl/rxn[1]	20 µl/rxn[1]	Final conc.
HOT FIREPol® Probe Universal qPCR Mix	2 µl	4 µl	1X
Forward Primer (10 µM)	0.2–0.4 µl	0.4–0.8 µl	200–400 nM
Reverse Primer (10 µM)	0.2–0.4 µl	0.4–0.8 µl	200–400 nM
Probe	x µl	x µl	100–250 nM

OPTIONAL: UNG	Follow supplier's recommendations for UNG treatment		
OPTIONAL: 100% DMSO	Variable	Variable	Variable
Template DNA (added at step 4)	Variable	Variable	Variable[2]
Nuclease-free water	Up to 10 µl	up to 20 µl	
Total reaction volume	**10 µl**	**20 µl**	

[1] 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.

[2] Conc. of cDNA 0.1 pg/µl – 10 ng/µl; gDNA 10 pg/µl – 4 ng/µl

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
OPTIONAL: UNG treatment[1]	Follow supplier's recommendations for UNG treatment		1
Initial activation[2]	**95°C**	**10 min**	1
Denaturation	95°C	15–20 sec	40

Annealing/Elongation	60	60 sec	

[1] OPTIONAL! Add UNG treatment step only if UNG enzyme is added in the reaction mix for carryover-contamination removal. Follow the vendor's guidelines.

[2] Incubation at 95°C for 10 minutes is crucial for the full activation of HOT FIREPol® DNA Polymerase.

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

Use dedicated software, such as Primer3 (bioinfo.ut.ee/primer3) or NCBI Primer-BLAST (ncbi.nlm.nih.gov/tools/primer-blast) to design target-specific primers and probes. Analyze your primers and probes for self-complementarity and stable secondary structures (e.g. hairpins) in their sequences. Avoid the 3'-self complementarity, because it increases the probability of primer-dimers formation. GC-content of primers and probe(s) should range from 35–65%.

In case of multiplexing, test the performance of primer–probe sets in individual assays before combining them in a multiplex assay.

Check the qPCR cycler's user manual for correct setup for multiplex analysis.

Primers

1. The optimal primer length is 20–30 bp targeting amplicons of 50–150 base pairs. Avoid runs of more than four consecutive G or C bases.

2. The optimal melting temperature (Tm) of the primers is 60–64°C. In order for both primers to bind efficiently, Tm of the two primers should not differ by more than 3°C.

Probe

1. The length of the probes should be 9–40 bases. For the hydrolysis probes, it is usually up to 30 bases. The probe binding site should be in close proximity to the forward or reverse primer. However, the probe and primer binding sites should not overlap. Probe can bind to either strand of the target.

2. Melting temperature (Tm) of the probe should be 6–8°C higher than the Tm of the primers. In case the Tm of the probe is too low, less of the probe is bound to the target sequence. As a consequence, the product may be amplified, but the sensitivity will be compromised, because not all target sites are occupied by the probe, which results in reduced fluorescence signal and not a true representation of the target amount in the sample.

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. Use up to 50 ng of complex (e.g. eukaryotic) DNA and up to 100 ng of cDNA in your reactions.

3. The final amount of cDNA sample in the qPCR reaction is up to one tenth of the final reaction volume. Overload of cDNA sample may inhibit your qPCR.

4. Perform and analyze your qPCR reactions in triplicates on a serially diluted template (e.g. 10-fold dilution series). 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.

5. To monitor possible contamination and primer-dimer formation, always include a no-template control (NTC), replacing the DNA template with nuclease-free water.

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-08-88 v1. Effective from: 31.01.2024

Reason for revision: product name and catalogue number updated: former HOT FIREPol® Probe Universal qPCR Mix, 5x (Cat. No.: 08-17-0000S, 08-17-00001, 08-17-00001-5, 08-17-00020).

*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. The 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. 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. **Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

Trademark information: FIREPol is a registered trademark of Solis BioDyne OÜ.

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