

## HOT FIREPoI<sup>®</sup> Probe Universal qPCR Mix, 5x

Cat. No.	Pack Size	20 µl rxn
08-17-0000S	0.2 ml	50
08-17-00001	1 ml	250
08-17-00001-5	5 x 1 ml	1250
08-17-00001-10	10 x 1 ml	2500
08-17-00008	8 ml	2000
08-17-00020	20 ml	5000

For *in vitro* use only

### Description:

HOT FIREPoI<sup>®</sup> Probe Universal qPCR Mix is optimized for real-time quantitative PCR assays and contains all the components necessary to perform singleplex or duplex qPCR, with the exception of template, primers, and probes. The qPCR Mix contains optimized components and HOT FIREPoI<sup>®</sup> DNA Polymerase supplied in a proprietary reaction buffer that enables efficient amplification of regular and GC-rich targets.

HOT FIREPoI<sup>®</sup> Probe Universal qPCR Mix is optimized for DNA/LNA hydrolysis probes based on the 5' flap endonuclease activity.

HOT FIREPoI<sup>®</sup> DNA Polymerase 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.

### Applications:

- DNA/LNA hydrolysis probe-based assays
- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination

### Benefits:

- Increased sensitivity and specificity for a wide range of templates, including AT-rich, GC-rich and regular cDNA and gDNA.
- Suitable for singleplex and duplex assays.
- Reaction set-up at room temperature
- Wide instrument compatibility: suitable for qPCR cyclers regardless of ROX requirements (except capillary).

### Mix Composition:

- **HOT FIREPoI<sup>®</sup> DNA Polymerase**
- **5x Probe Universal qPCR buffer**
- **15 mM MgCl<sub>2</sub>**  
*1x PCR solution – 3 mM MgCl<sub>2</sub>*
- **dNTPs**, including dUTP  
*The mix allows UNG treatment to prevent carryover contamination from previous PCR runs. IMPORTANT: UNG is not included in the HOT FIREPoI<sup>®</sup> Probe Universal qPCR Mix and should be purchased separately.*
- **Internal reference based on ROX dye**  
*The dye is used to normalize the fluorescent reporter signal generated in qPCR. The product is compatible with both low ROX and high ROX system requirements. For multiplex application: if ROX dye is used as one of the fluorophores, internal reference might interfere with the signal.*

### Reagents provided with the mix in a separate vial:

- **100% DMSO**

### Shipping and Storage conditions:

Routine storage: -18°C to -28°C

Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of the product.

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

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

**Recommended qPCR reaction mix:**

Component	Volume	Final conc.
HOT FIREPol® Probe Universal qPCR Mix (5x)	4 µl	1x
Forward primer (10 µM)	0.4–0.8 µl	200–400 nM
Reverse primer (10 µM)	0.4–0.8 µl	200–400 nM
Probe	x µl	100–250 nM
OPTIONAL: UNG <sup>1</sup> (Uracil-N-glycosylase)	Variable	Variable <sup>1</sup>
OPTIONAL: 100% DMSO <sup>2</sup>	Variable	Up to 10%
DNA template	Variable	Variable <sup>3</sup>
H <sub>2</sub> O PCR grade	up to 20 µl	
<b>Total</b>	<b>20 µl</b>	

<sup>1</sup> Please add UNG according to manufacturer's specification.

<sup>2</sup> DMSO is recommended as a PCR additive for templates with high GC content. In some cases, DMSO is also required to relax secondary structures. While testing it is recommended to include one sample with additional 2.5 % DMSO to test if it improves the results. For further DMSO optimization the concentration can be raised in 2.5% increments up to 10% based on the table below. Volumes are given per 20 µl final reaction volume. The highest DMSO concentration recommended is 10% which should be used for all templates with GC content over 70%.

<sup>3</sup> Conc. of cDNA 0.1 pg/µl–10 ng/µl; gDNA 10 pg/µl–4 ng/µl

Final DMSO concentration	2.5%	5%	7.5%	10%
Additional volume of 100% DMSO	0.5 µl	1 µl	1.5 µl	2 µl

**Recommended qPCR cycling protocol:**

Cycle step	Temp.	Time	Cycles
OPTIONAL: UNG treatment <sup>4</sup>	Variable <sup>4</sup>	Variable <sup>4</sup>	1
<b>Initial activation<sup>5</sup></b>	<b>95°C</b>	<b>10 min</b>	1
Denaturation	95°C	15–20 s	40
Annealing/Extension <sup>6</sup>	60°C	60 s	

<sup>4</sup> **OPTIONAL!** Add UNG treatment step **ONLY** if UNG enzyme is added in the reaction mix for carryover contamination removal. Use UNG according to manufacturer's specification.

<sup>5</sup> To activate the polymerase, include an incubation step **at 95°C for 10 minutes** at the beginning of the qPCR cycle.

<sup>6</sup> The annealing temperature (Ta) depends on the melting temperature (Tm) of the primers. A Ta that is about 2 to 5°C lower than the Tm of the primers is generally suitable. Performing temperature gradient is recommended.

**Safety warnings and precautions:**

This product and its components should be handled only by persons trained in laboratory techniques. It is advisable to wear suitable protective clothing, such as laboratory overalls, gloves and safety glasses. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water. 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](mailto:support@solisbiodyne.com)

Online chat is available at [www.solisbiodyne.com](http://www.solisbiodyne.com)

DS-08-17 v2

Revised 18.06.2021

**Permitted Use:** This product is supplied for research use only (the **Permitted Use**). If the customer wishes to use the product for any purpose other than the Permitted Use, including (without limitation) resale or alteration, the customer should obtain the appropriate licence from Solis Biodyne. Some applications of this product may require a license/licenses from one or more third parties which are not provided by the purchase of this product. Users should obtain the licence if required. Covered by the patent EP2501716, made by the methods of US Patent No 9,321,999.

**Trademark information:** FIREPol® is an EU registered trademark of Solis BioDyne OÜ.

**Warranty and Disclaimer:** This product shall comply with its relevant specification and be fit for its stated purpose, but Solis BioDyne gives no other warranty and makes no representation as to description or quality. Any such warranty or representation is excluded, to the fullest extent permitted by law. In particular, but without limiting the foregoing, Solis BioDyne shall not be liable for the failure of the product to comply with its relevant specification where such failure arises as a result of: (i) customer negligence or because the customer failed to follow any of the applicable technical data or safety sheets, standard user materials, use guidelines or any other information provided by Solis BioDyne as to the storage, transportation, handling, use or maintenance of the products or other good practice regarding the same, or (ii) the customer altering the products in any way without the prior written consent from Solis BioDyne, or (iii) the products differing from the relevant specification as a result of changes made to ensure their compliance with applicable statutory or regulatory requirements.

Nothing shall limit or exclude Solis BioDyne's liability for death or personal injury caused by its negligence, fraud or fraudulent misrepresentation or any matter in respect of which it would be unlawful for Solis BioDyne to exclude or restrict liability. Without limiting the foregoing, Solis BioDyne shall under no circumstances whatever be liable to the customer, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of profit, or any indirect or consequential loss arising under or in connection with the products and Solis BioDyne's total liability to the customer in respect of all other losses arising under or in connection with the product, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed the price of the products supplied in respect of which the liability has arisen.

Solis BioDyne

Teaduspargi 9, 50411 Tartu, Estonia, tel: +372 740 9960, fax: +372 740 2079, e-mail: [info@solisbiodyne.com](mailto:info@solisbiodyne.com), [www.solisbiodyne.com](http://www.solisbiodyne.com)