SOLIS BIODYNE

Data Sheet

HOT FIREPol® 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
08-17-00020	20 ml	5000

For *in vitro* use only

Description:
HOT FIREPol® 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 FIREPol® DNA Polymerase supplied in a proprietary reaction buffer that enables efficient amplification of regular and GC-rich targets.
HOT FIREPol® Probe Universal qPCR Mix is optimized for DNA/LNA hydrolysis probes based on the 5' flap endonuclease activity.
HOT FIREPol® 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 FIREPol® DNA Polymerase**
- **5x Probe Universal qPCR buffer**
- **15 mM $MgCl_2$**
  1x PCR solution – 3 mM $MgCl_2$
- **dNTPs**, including dUTP
  The mix allows UNG treatment to prevent carryover contamination from previous PCR runs.
  IMPORTANT: UNG is not included in the HOT FIREPol® 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: -18ºC to -28ºC
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.

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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[1] (Uracil-N-glycosylase)	Variable	Variable[1]
OPTIONAL: 100% DMSO[2]	Variable	Up to 10%
DNA template	Variable	Variable[3]
H_2O PCR grade	up to 20 µl	
Total	**20 µl**	

[1] Please add UNG according to manufacturer's specification.

[2] 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%.

[3] 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[4]	Variable[4]	Variable[4]	1
Initial activation[5]	**95ºC**	**10 min**	1
Denaturation	95ºC	15–20 s	40
Annealing/Extension[6]	60ºC	60 s	

[4] **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.

[5] To activate the polymerase, include an incubation step **at 95ºC for 10 minutes** at the beginning of the qPCR cycle.

[6] 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

Online chat is available at www.solisbiodyne.com

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