Solis BioDyne
Data Sheet

5x HOT FIREPol® Probe Universal qPCR Mix

Suitable for ROX-dependent and ROX-independent qPCR cyclers

Cat. No.	Pack Size	Conc. ($MgCl_2$)
08-17-0000S	0.2 ml SAMPLE (50 reactions)	15 mM
08-17-00001	1 ml (250 reactions)	15 mM
08-17-00008	8 ml (2000 reactions)	15 mM
08-17-00020	20 ml (5000 reactions)	15 mM

For *in vitro* use only

Description:

5x 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.

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 – the mix is stable at ambient temperature for one month.**
- **Benchtop stability for 48 hours for pre-assembled reactions.**
- **Wide instrument compatibility: suitable for qPCR cyclers regardless of ROX requirements (except capillary).**

Applications:

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

Shipping and Storage conditions:

Routine storage: -20ºC
Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of HOT FIREPol® Probe Universal qPCR Mix.

Mix Composition:

- **HOT FIREPol® DNA Polymerase**
- **5x HOT FIREPol® 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 runs.
  IMPORTANT: UNG is not included in the 5x HOT FIREPol® Probe Universal qPCR Mix and must be purchased separately.
- **Internal reference based on ROX dye**
  For multiplex application: if ROX dye is used as one of the fluorophores, internal reference might interfere with the signal – a version without ROX is available upon request

In a separate vial:

- ***100% DMSO** is included in the kit in a separate vial. 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 following table. Volumes are given per reaction depending on final volume of reaction mix. The highest DMSO concentration recommended is 10% which should be used for all templates with GC content over 70%*

Addition of 100% DMSO

Final conc. of DMSO	10 µl/rxn	20 µl/rxn
2,5 %	0.25 µl	0.5 µl
5%	0.5 µl	1 µl
7,5%	0.75 µl	1.5 µl
10%	1 µl	2 µl

Recommended qPCR reaction mix:

Component	10 µl/rxn	20 µl/rxn	Final conc.
5x HOT FIREPol® Probe Universal qPCR Mix	2 µl	4 µl	1x
Primer Forward (10 pmol/µl)	0.2-0.4 µl	0.4-0.8 µl	200-400 nM
Primer Reverse (10 pmol/µl)	0.2-0.4 µl	0.4-0.8 µl	200-400 nM
Probe	x µl	x µl	100-250 nM
OPTIONAL: UNG (Uracil-N-glycosylase)	x µl	x µl	x U/µl
OPTIONAL: 100% DMSO	variable	variable	Up to 10%
DNA template [1]	Variable[1]	Variable[1]	Variable[1]
H_2O PCR grade	up to 10 µl	up to 20 µl	
Total	**10 µl**	**20 µl**	

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

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Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
OPTIONAL: UNG treatment[2]	**50ºC**	**2 min**	1
Initial activation[3]	**95ºC**	**10 min**	1
Denaturation	95ºC	15-20 s	40
Annealing/Elongation	60ºC	60 s	

[2] **OPTIONAL!** Add UNG treatment step ONLY if UNG enzyme is added in the reaction mix for carryover contamination removal.

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

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.