Solis BioDyne
Data Sheet

5x HOT FIREPol® Probe GC 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:

HOT FIREPol® Probe GC qPCR Mix is optimized for real-time quantitative PCR assays and contains all the components necessary to perform simplex or duplex qPCR, with the exception of template, primers, and probe. The qPCR Mix contains optimized components and HOT FIREPol® DNA Polymerase supplied in a proprietary reaction buffer that enables efficient amplification of GC-rich targets.

HOT FIREPol® Probe GC qPCR Mix is optimized for DNA/LNA hydrolysis probes based on the 5´–3´ exonuclease 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 wide range of templates, including GC-rich cDNA and gDNA (up to 75%)**
- **Suitable for singleplex and duplex assays.**
- **Reaction set-up at room temperature – mix is stable at ambient temperature for one month**
- **Benchtop stability for 48 hours for pre-assambled reactions.**
- **Wide instrument compatibility: mix is compatible with any real-time instrument other than 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 GC qPCR Mix.

Mix Composition:

- **HOT FIREPol® DNA Polymerase**
- **5x Probe GC qPCR buffer**
- **15 mM $MgCl_2$**
  1x PCR solution – 3 mM $MgCl_2$
- **dNTPs**, including dUTP
  Mix may used with UNG to prevent carryover contamination from previous runs
- **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 – version without ROX available upon request

In separate vial:

- **100% DMSO**
  DMSO is recommended as a PCR additive for high GC content templates. In some cases DMSO is also required to relax secondary structures. Concentration of DMSO should be varied from 2% in 2% increments. Highest DMSO concentration recommended is 10% which should be used in all templates with GC content over 70%.

Recommended qPCR reaction mix:

Component	Volume	Final conc.
HOT FIREPol® Probe GC qPCR Mix	4 µl	1x
100% DMSO	variable	Up to 10%
Primer Forward (10 pmol/µl)	0.4-0.8 µl	200-400 nM
Primer Reverse (10 pmol/µl)	0.4-0.8 µl	200-400 nM
Probe	x µl	100-250 nM
DNA template	1-5 µl	0.001-2 ng/µl
H_2O PCR grade	up to 20 µl	
Total	**20 µl**	

Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
Initial activation*	**95ºC**	**10 min**	1
Denaturation	95ºC	15-20 s	40
Annealing/Elongation	60ºC	60 s	

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

In order to prevent contamination, we recommend you to setup the reaction under laminar or in PCR box.

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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.

Some applications this product is used in may require a license which is not provided by the purchase of this product. Users should obtain the license if required

OPTIONAL – UNG TREATMENT

Reaction mix in case of additional UNG treatment:

Component	Volume	Final conc.
HOT FIREPol® Probe GC qPCR Mix	4 µl	1x
100% DMSO*	variable	Up to 10%
Primer Forward (10 pmol/µl)	0.2-0.4 µl	100-200 nM
Primer Reverse (10 pmol/µl)	0.2-0.4	100-200 nM
UNG (Uracil-N-glycosylase)	x µl[1]	x
DNA template	x µl	0.001-2 ng/µl
H_2O PCR grade	up to 20 µl	
Total	**20 µl**	

[1] Please add UNG according to manufacturer's specification. UNG is not included in the mix and must be purchased separately.

qPCR cycles in case of additional UNG treatment:

Cycle step	Temp.	Time	Cycles
UNG treatment	50ºC	2 min	1
Initial denaturation*	**95ºC**	**10 min**	1
Denaturation	95ºC	15-20 s	40
Annealing/Elongation	60º	60 s	

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

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