SOLIS BIODYNE

Data Sheet

HOT FIREPol® Multiplex qPCR Mix (ROX), 5x

Cat. No.	Pack Size	20 µl rxn
08-02-0000S	0.2 ml	50
08-02-00001	1 ml	250
08-02-00001-5	5 x 1 ml	1250
08-02-00001-10	10 x 1 ml	2500
08-02-00020	20 ml	5000

For *in vitro* use only

Description:
HOT FIREPol® Multiplex qPCR Mix is optimized for amplifying up to 4 targets in a single reaction in real-time quantitative PCR assays. The qPCR Mix comprises all the components necessary (except primers, probes and template) to perform qPCR: HOT FIREPol® DNA Polymerase, optimized buffer components, ultrapure dNTPs and $MgCl_2$ and ROX passive reference dye according to system requirements.

HOT FIREPol® Multiplex qPCR Mix (ROX) is optimized for DNA 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:
- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Viral load determination

Mix Composition:
- **HOT FIREPol® DNA Polymerase**
- **5x Multiplex 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 HOT FIREPol® Multiplex 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.
  If ROX, Texas Red or similar dye is used as one of the fluorophores, ROX passive reference dye might interfere with the signal. A version without passive reference or with Purple reference dye is available.

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.

Recommendations:
Reaction setup at room temperature.
In order to prevent contamination, we recommend you to setup the reaction under laminar or in PCR box.

Recommended qPCR reaction mix:

Component	Volume	Final conc.
HOT FIREPol® Multiplex qPCR Mix (ROX) (5x)	4 µl	1x
Forward primer (10 µM)	0.4–0.8 µl	200–400 nM (each)
Reverse primer (10 µM)	0.4–0.8 µl	200–400 nM (each)
Probe	x µl	100–250 nM (each)
OPTIONAL: UNG (Uracil-N-glycosylase)	Variable	Variable[1]
DNA template	Variable	Variable[2]
H_2O PCR grade	up to 20 µl	
Total	**20 µl**	

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

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

Recommended qPCR cycling protocol:

Cycle step	Temp.	Time	Cycles
OPTIONAL: UNG treatment[3]	Variable[3]	Variable[3]	1
Initial activation[3]	**95ºC**	**10 min**	1
Denaturation	95ºC	15–20 s	40
Annealing/Extension[4]	60ºC	60 s	

[3] **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.

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

[4] 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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