

Data Sheet

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

Cat. No.	Pack Size	20 µl rxn
08-03-0000S	0.2 ml	50
08-03-00001	1 ml	250
08-03-00001-5	5 x 1 ml	1250
08-03-00001-10	10 x 1 ml	2500
08-03-00020	20 ml	5000

For *in vitro* use only

Description:

HOT FIREPol® Multiplex qPCR Mix (Purple) 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 MgCl₂, Purple passive reference dye according to system requirements

HOT FIREPol® Multiplex qPCR Mix (Purple) 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 MgCl₂**
1x PCR solution – 3 mM MgCl₂
- **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.
- **Purple dye**
Purple dye is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR. If Cy5 or similar dye is used as one of the fluorophores, Purple passive reference dye might interfere with the signal. A version without passive reference or with ROX reference dye is available.

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.

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 (Purple) (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 ₂ O 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	Cycle s
OPTIONAL: UNG treatment ³	Variable ³	Variable ³	1
Initial activation³	95°C	10 min	1
Denaturation	95°C	15–20 s	40
Annealing/Extension ⁴	60°C	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 95°C for 10 minutes** at the beginning of the qPCR cycle.

⁴ The annealing temperature (T_a) depends on the melting temperature (T_m) of the primers. A T_a that is about 2 to 5°C lower than the T_m of the primers is generally suitable. Performing temperature gradient is recommended.

Cycler compatibility:

HOT FIREPo[®] Multiplex qPCR Mix (Purple) is compatible with qPCR cyclers, such as Applied Biosystems™ 7500 Fast Real-Time PCR System, 7500 Real-Time PCR System, ViiA™ 7 PCR System, and QuantStudio™ 6 Flex, 7 Flex, and 12K Flex Real-Time PCR systems, which enable using Mustang Purple™ channel for reference signal normalization.

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

DS-08-03 v3
Revised 12.04.2022

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