

5x HOT FIREPol® Multiplex qPCR Mix (ROX)

Cat. No.	Pack Size	Conc. (MgCl ₂)
08-02-0000S	0.2 ml SAMPLE (50 reactions)	15 mM
08-02-00001	1 ml (250 reactions)	15 mM
08-02-00008	8 ml (2000 reactions)	15 mM
08-02-00020	20 ml (5000 reactions)	15 mM

For *in vitro* use only

Description:

HOT FIREPol® Multiplex qPCR Mix (ROX) is optimized for amplifying multiple 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, MgCl₂ 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 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 5x HOT FIREPol® Multiplex qPCR Mix (ROX) and must be purchased separately.
- **ROX dye**
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.

ROX Dye:

ROX dye is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR.

Recommendations:

Reaction setup at room temperature is recommended for HOT FIREPol® Multiplex qPCR Mix (ROX).

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.
5x HOT FIREPol® Multiplex qPCR Mix (ROX)	4 µl	1x
Primer Forward (10 pmol/µl)	0.4-0.8 µl	200-400 nM (each)
Primer Reverse (10 pmol/µl)	0.4-0.8 µl	200-400 nM (each)
Probe	x µl	100-250 nM (each)
OPTIONAL: UNG (Uracil-N-glycosylase)	Follow supplier recommendations for UNG treatment.	
DNA template ¹	variable ¹	variable ¹
H ₂ O PCR grade	up to 20 µl	
Total	20 µl	

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

Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
OPTIONAL: UNG treatment²	Follow supplier recommendations for UNG treatment.		1
Initial activation³	95°C	10 min	1
Denaturation	95°C	15-20 s	40
Annealing/Elongation	60°C	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 **95°C for 10 minutes** at the beginning of the qPCR cycle.

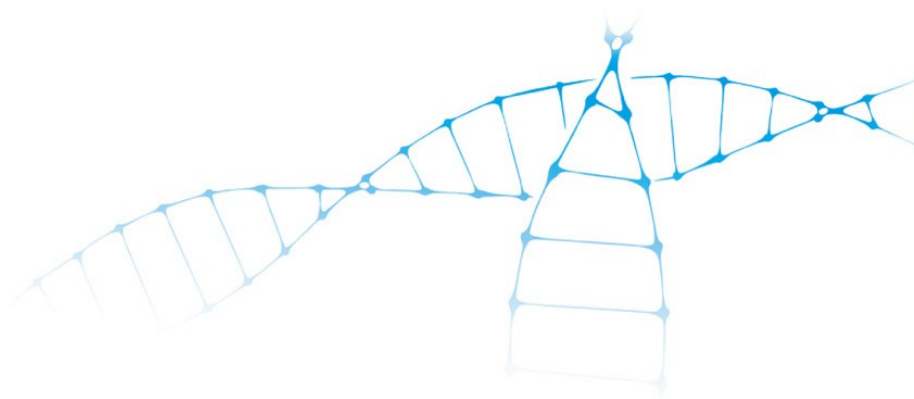
Shipping and Storage conditions:

Routine storage: -20°C

Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of HOT FIREPol® Multiplex qPCR Mix (ROX).

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.



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