



## 5x HOT FIREPol® Probe qPCR Mix Plus (ROX)

Cat. No.	Pack Size	Conc. (MgCl <sub>2</sub> )
08-14-0000S	0.2 ml SAMPLE (50 reactions)	15 mM
08-14-00001	1 ml (250 reactions)	15 mM
08-14-00008	8 ml (2000 reactions)	15 mM
08-14-00020	20 ml (5000 reactions)	15 mM

For *in vitro* use only

### Description:

HOT FIREPol® Probe qPCR Mix Plus (ROX) is optimized for real-time quantitative PCR assays and contains all the components necessary to perform 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 detection of low copy number targets. HOT FIREPol® Probe qPCR Mix Plus (ROX) is optimized for DNA hydrolysis probes based on the 5' flap endonuclease activity.

HOT FIREPol® DNA Polymerase is activated by a 12 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 Probe qPCR buffer**
- **15 mM MgCl<sub>2</sub>**  
*1x PCR solution – 3 mM MgCl<sub>2</sub>*
- **dNTPs**
- **ROX dye**

### ROX Dye:

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

### Recommended qPCR reaction mix:

Component	Volume	Final conc.
5x HOT FIREPol® Probe qPCR Mix Plus	4 µl	1x
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 <sup>1</sup>	variable <sup>1</sup>	variable <sup>1</sup>
H <sub>2</sub> O PCR grade	up to 20 µl	
<b>Total</b>	<b>20 µl</b>	

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

### Recommended qPCR cycles:

Cycle step	Temp.	Time	Cycles
<b>Initial activation<sup>2</sup></b>	<b>95°C</b>	<b>12 min</b>	1
Denaturation	95°C	15-20 s	40
Annealing/Elongation	60°C	60 s	

<sup>2</sup> To activate the polymerase, include an incubation step **at 95°C for 12 minutes** at the beginning of the qPCR cycle.

### Recommendations:

Reaction setup at room temperature is highly recommended for HOT FIREPol® Probe qPCR Mix Plus.

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

### 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® Probe qPCR Mix Plus (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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