



5x HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX)

Cat. No.	Pack Size	Conc. (MgCl ₂)
08-24-0000S	0.2 ml SAMPLE (50 reactions)	12.5 mM
08-24-00001	1 ml (250 reactions)	12.5 mM
08-24-00008	8 ml (2000 reactions)	12.5 mM
08-24-00020	20 ml (5000 reactions)	12.5 mM

For *in vitro* use only

Description:

HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) is an optimised ready-to-use solution for real-time quantitative PCR assays, incorporating EvaGreen® dye. It comprises all the components necessary to perform qPCR: HOT FIREPol® DNA Polymerase, ultrapure dNTPs, MgCl₂, EvaGreen® dye and ROX dye according to system requirements. The user simply needs to add water, template and primers.

HOT FIREPol® DNA Polymerase is activated by a 15 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 EvaGreen® qPCR buffer**
- **12.5 mM MgCl₂**
1x PCR solution – 2.5 mM MgCl₂
- **dNTPs**
- **EvaGreen® dye**
- **ROX dye**

EvaGreen® Dye:

EvaGreen® is a DNA-binding dye with many features that make it a superior alternative to SYBR® Green I for qPCR. Apart from having similar spectra, EvaGreen® has three important features that set it apart from SYBR® Green I: EvaGreen® has much less PCR inhibition, is an extremely stable dye and has been shown to be non-mutagenic and non-cytotoxic. EvaGreen® is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM!

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® EvaGreen® qPCR Mix Plus	4 µl	1x
Primer Forward (10 pmol/µl)	0.16-0.5 µl	80-250 nM
Primer Reverse (10 pmol/µl)	0.16-0.5 µl	80-250 nM
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
Initial activation²	95°C	15 min	1
Denaturation	95°C	15 s	40
Annealing	60°-65°C	20 s	
Elongation	72°C	20 s	

² To activate the polymerase, include an incubation step at **95°C for 15 minutes** at the beginning of the qPCR cycle.

Recommendations:

Reaction setup at room temperature is highly recommended for HOT FIREPol® EvaGreen® 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® EvaGreen® 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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