



## 5x HOT FIREPol® EvaGreen® HRM Mix (ROX)

Cat. No.	Pack Size	Conc. (MgCl <sub>2</sub> )
08-33-0000S	0.2 ml SAMPLE (50 reactions)	12.5 mM
08-33-00001	1 ml (250 reactions)	12.5 mM
08-33-00008	8 ml (2000 reactions)	12.5 mM
08-33-00020	20 ml (5000 reactions)	12.5 mM

For *in vitro* use only

### Description:

5x HOT FIREPol® EvaGreen® HRM Mix is an optimised ready-to-use solution for High Resolution Melt (HRM) Analysis, incorporating EvaGreen® dye. It comprises all the components necessary to perform qPCR and HRM Analysis: HOT FIREPol® DNA Polymerase, ultrapure dNTPs, MgCl<sub>2</sub>, 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 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:

- High Resolution Melt (HRM)

### Mix Composition:

- **HOT FIREPol® DNA Polymerase**
- **5x EvaGreen® HRM buffer**
- **12.5 mM MgCl<sub>2</sub>**  
*1 x PCR solution – 2.5 mM MgCl<sub>2</sub>*
- **dNTPs**
- **EvaGreen® dye**
- **BSA**
- **ROX dye**

### EvaGreen® Dye:

EvaGreen® is a DNA-binding dye with many features that make it a superior for HRM. 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!

### 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® HRM Mix.

### Recommended qPCR reaction mix:

Component	Volume	Final conc.
5x HOT FIREPol® EvaGreen® HRM Mix	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 <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 s	40
Annealing	60°-65°C	20 s	
Elongation	72°C	20 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.

For HRM step please use settings recommended by producer

### 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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