



## 5x HOT FIREPol® MultiPlex Mix Ready to Load

with 10 mM MgCl<sub>2</sub>

for amplification of up to 20 targets in a single reaction

Cat. No.	Pack Size	Conc. (MgCl <sub>2</sub> )
04-36-00S20	0,1 ml SAMPLE (25 rxn)	10 mM
04-36-00120	1 ml (250 rxn)	10 mM
04-36-02020	20 ml (5000 rxn)	10 mM

For *in vitro* use only

### Description:

5x HOT FIREPol® MultiPlex Mix is a premixed ready-to-use solution containing all reagents required for hot-start multiplex PCR (except template, primers and water) and an additional compound needed for direct loading onto agarose gel and two tracking dyes (blue and yellow) that allow to monitor progress during electrophoresis.

### Applications:

- Hot Start PCR
- Multiplex PCR

### Mix Composition:

- **HOT FIREPol® DNA polymerase**
- **5x MultiPlex Buffer**
- **10 mM MgCl<sub>2</sub>**  
*1x PCR solution – 2mM MgCl<sub>2</sub>*
- **dNTPs**
- **BSA**
- **Blue dye**  
*Migration equivalent to 3.5-4.5 kb DNA fragment*
- **Yellow dye**  
*Migration equivalent to 35-45 bp DNA fragment*
- **Compound that increases sample density for direct loading**

### Shipping and Storage conditions:

Routine storage: -20°C

Shipping and temporary storage for up to 1 month at room temperature or storage for up to 6 months at 2-8°C has no detrimental effects on the quality of 5x HOT FIREPol® MultiPlex Mix.

### Recommendations:

Reaction setup at room temperature is highly recommended for HOT FIREPol® MultiPlex Mix.

5x HOT FIREPol® MultiPlex Mix is not recommended for use in applications where spectro-photometric measurements (absorbance or fluorescence) are necessary because yellow and blue dyes can interfere with these applications.

### Recommended PCR reaction mix:

Component	Volume	Final conc.
5x HOT FIREPol® MultiPlex Mix	4µl	1x
Forward primer (10 pmol/µl)	0.2-0.6µl	0.1-0.3 µM (each)
Reverse primer (10 pmol/µl)	0.2-0.6µl	0.1-0.3 µM (each)
Template DNA	Variable <sup>1</sup>	Variable <sup>1</sup>
H <sub>2</sub> O	Up to 20 µl	

<sup>1</sup>Conc. of cDNA 0.01 pg/µl -0.1 ng/µl ; gDNA 0.1 ng/µl – 10ng/µl

### Recommended PCR cycles:

Operation	Temp.	Time	Cycles
<b>Initial activation*</b>	<b>95°C</b>	<b>12 min</b>	1
Denaturation	95°C	20-30 s	25-30
Annealing	58-62°C	30-60 s	
Elongation	72°C	30 s - 3 min	
Final elongation	72°C	5-10 min	1

\* To activate the polymerase, include an incubation step at **95°C for 12 minutes** at the beginning of the PCR cycle.

### Troubleshooting:

For troubleshooting the following additives may be included with your order free of charge upon request:

- **25 mM MgCl<sub>2</sub>** (cat. no. 05-11-00025) can be used for optimization of the MgCl<sub>2</sub> concentration. The 1x PCR mix solution of 2 mM MgCl<sub>2</sub> gives satisfactory results in most applications. If necessary the MgCl<sub>2</sub> concentration may be increased in 0,25-1 mM increments up to 5 mM concentration.
- **10x GC-rich Enhancer** (cat. no. 05-16-00010) can be used for optimization with GC-rich templates. It modifies the melting behavior of nucleic acids and enhances the amplification of regions with secondary structures and high GC-content. 10x GC-rich Enhancer should be used at a defined working concentration (1x, 2x or 3x solution) and only if non-specific amplification occurs.
- **100% DMSO** is recommended as a PCR additive for templates with high GC content. In some cases DMSO is also required to relax secondary structures. For optimization DMSO concentration can be raised in 2,5% increments up to 10%.

### 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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