



5x HOT FIREPol® MultiPlex Mix

with 10 mM MgCl₂

for amplification of up to 20 targets in a single reaction

| Cat. No. | Pack Size | Conc. (MgCl ₂) |
|-------------|---------------------------|----------------------------|
| 04-34-00S20 | 0,1 ml SAMPLE (25 rxn) | 10 mM |
| 04-34-00120 | 1 ml (250 rxn) | 10 mM |
| 04-34-02020 | 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).

Applications:

- Hot Start PCR
- Multiplex PCR

Mix Composition:

- **HOT FIREPol® DNA polymerase**
- **5x MultiPlex Buffer**
- **10 mM MgCl₂**
1x PCR solution – 2mM MgCl₂
- **dNTPs**
- **BSA**
- **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.

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 ¹ | variable ¹ |
| H ₂ O | Up to 20 µl | |

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

Recommended PCR cycles:

| Operation | Temp. | Time | Cycles |
|----------------------------|-------------|---------------|--------|
| Initial activation* | 95°C | 12 min | 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 MgCl₂** (cat. no. 05-11-00025) can be used for optimization of the MgCl₂ concentration. The 1x PCR mix solution of 2 mM MgCl₂ gives satisfactory results in most applications. If necessary the MgCl₂ 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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