



5x HOT FIREPol® Blend Master Mix

With 10 mM MgCl₂

| Cat. No. | Pack Size | Conc. (MgCl ₂) |
|-------------|---------------------------------|----------------------------|
| 04-27-00S20 | 0.1 ml SAMPLE (25 reactions) | 10 mM |
| 04-27-00120 | 1 ml (250 reactions) | 10 mM |
| 04-27-02020 | 20 ml (5000 reactions) | 10 mM |

For *in vitro* use only

Description:

5x HOT FIREPol® Blend Master Mix is a premixed ready-to-use solution containing all reagents required for PCR except template, primers and water.

HOT FIREPol® Blend Master Mix contains two carefully optimized enzymes – HOT FIREPol® DNA polymerase and a proofreading polymerase. This enzyme blend has both the 5' flap endonuclease activity as well as the 3'→5' proofreading activity. HOT FIREPol® Blend Master Mix exhibits an increased fidelity (up to five fold) compared to HOT FIREPol®. Generated PCR products are compatible with blunt-end and TA cloning procedures (to increase the blunt end cloning efficiency treat the PCR products with T4 DNA polymerase or DNA polymerase I large Klenow fragment prior to cloning).

Applications:

- Hot Start PCR

Mix Composition:

- **HOT FIREPol® DNA polymerase**
- **Proofreading enzyme**
- **5x Blend Master Mix Buffer**
- **10 mM MgCl₂**
1x PCR solution – 2 mM MgCl₂
- **1 mM dNTPs of each**
1x PCR solution – 200 μM dATP, 200 μM dCTP, 200 μM dGTP and 200 μM dTTP
- **BSA**

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® Blend Master Mix.

Recommendations:

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

We recommend using 5x HOT FIREPol® Blend Master Mix in any PCR application that will be visualized by agarose gel electrophoresis and ethidium bromide staining.

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

Recommended PCR reaction mix:

| Component | Volume | Final conc. |
|----------------------------------|-----------------------|-----------------------|
| 5x HOT FIREPol® Blend Master Mix | 4 μl | 1 x |
| Forward primer (10 pmol/μl) | 0.2-0.6 μl | 0.1-0.3 μM |
| Reverse primer (10 pmol/μl) | 0.2-0.6 μl | 0.1-0.3 μM |
| DNA template ¹ | variable ¹ | variable ¹ |
| Add H ₂ O | Up to 20 μl | |

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

Recommended PCR cycles:

| Operation | Temp. | Time | Cycles |
|---------------------------------------|-------------|------------------|--------|
| Initial activation² | 95°C | 12-15 min | 1 |
| Denaturation | 95°C | 10-20 s | 25-30 |
| Annealing | 54-66°C | 30-60 s | |
| Elongation | 72°C | 20 s - 4 min | |
| Final elongation | 72°C | 5-10 min | |

²To activate the polymerase, include an incubation step at **95°C for 12-15 minutes** at the beginning of the PCR cycle.

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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