



5x HOT FIREPol® Blend Master Mix Ready to Load

With 12.5 mM MgCl₂

Cat. No.	Pack Size	Conc. (MgCl ₂)
04-25-00S25	0.1 ml SAMPLE (25 reactions)	12.5 mM
04-25-00125	1 ml (250 reactions)	12.5 mM
04-25-02025	20 ml (5000 reactions)	12.5 mM

For *in vitro* use only

Description:

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

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**
- **12.5 mM MgCl₂**
1x PCR solution – 2.5 mM MgCl₂
- **1 mM dNTPs of each**
1x PCR solution – 200 μM dATP, 200 μM dCTP, 200 μM dGTP and 200 μM dTTP
- **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® Blend Master Mix Ready to Load.

Recommendations:

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

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

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 Ready to Load	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
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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