



5x FIREPol® Master Mix Ready to Load (12.5 mM MgCl₂)

Cat. No.	Pack Size	Conc. (MgCl ₂)
04-12-00S25	0.1 ml SAMPLE (25 reactions)	12.5 mM
04-12-00125	1 ml (250 reactions)	12.5 mM

For *in vitro* use only

Description:

5x FIREPol® 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.

Applications:

- Suited for a wide range of PCR assays

Reagent Composition:

- **FIREPol® DNA polymerase**
- **5x Reaction Buffer B**
0.4 M Tris-HCl, 0.1 M (NH₄)₂SO₄, 0.1% w/v Tween-20
- **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
- **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 FIREPol® Master Mix Ready to Load.

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.

Recommended PCR reaction mix:

Component	Volume	Final conc.
5x FIREPol® 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	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 denaturation	95°C	3-5 min	1
Denaturation	95°C	15-30 s	25-30
Annealing	54-66°C	30-60 s	
Elongation ²	72°C	40 s - 4 min	
Final elongation	72°C	5-10 min	

²Elongation time should be ~1 min/1 kb.

Recommendations:

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

We do not recommend using the 5x FIREPol® Master Mix Ready to Load for PCR reactions where detergent free buffer system is required (detergent free 5 x Master Mix available upon request).

5x FIREPol® Master Mix Ready to Load is not recommended for use in applications where spectrophotometric 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.

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