5x FIREPol® Master Mix Ready To Load

Technical Data Sheet

Catalogue	Pack Size
FRTL-100	100 µL (25 x 20 µL PCR rxns)
FRTL-400	400 µL (100 x 20 µL PCR rxns)
FRTL-1000	1000 µL (250 x 20 µL PCR rxns)

For research and educational use only.

Description

A PCR master mix is a pre-prepared mixture that contains all of the components for a PCR reaction that are not sample-specific. You just need to add a DNA template, primers and water.

5x FIREPol® Master Mix Ready To Load is an optimised ready-to-use PCR master mix for routine PCR assays. This master mix contains thermostable Taq DNA polymerase FIREPol® , dNTPs, $MgCl_2$, and buffer.

Application Recommendations

Suitable for routine PCR

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

Suitable for room-temperature setup

Contains a superior room-temperature stable enzyme. However we recommend setting up relatively quickly or on ice if mispriming or primer dimer production is anticipated to be an issue.

Compatible with standard PCR instruments

5x FIREPol® Master Mix Ready to Load is compatible with standard PCR instruments, and works great as an alternative to other commercial Taq Polymerase Master Mixes.

For applications with post-PCR spectrophotometric measurements, cleanup is required

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. For example, it is not recommended for cycle sequencing reactions followed by enzymatic cleanup.

Not recommended for applications requiring detergent-free reagents

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Contains detergents, and therefore not recommended where detergents would interfere with consistent pipetting or experimental results, such as when using robots, microarrays, or denaturing high performance liquid chromatography.

Reagent Composition

- **FIREPol® DNA polymerase:** highly processive. thermostable DNA polymerase
- **5x Reaction Buffer B:** 0.4 M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 0.1% w/v Tween-20
- **7.5 mM $MgCl_2$:** 1x PCR solution contains 1.5 mM $MgCl_2$
- **1 mM dNTPs of each:** 1x PCR solution contains 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**

Storage & Stability

Routine storage at –20 ºC.

Temporary storage for up to 1 month at room temperature, or up to six months at 2 – 8 ºC, has no detrimental effects on the quality of FIREPol® Master Mix Ready to Load.

Shipping conditions

Shipped at room temperature.

Safety warnings and precautions

This product and its components are not considered hazardous in their given concentrations. However, as with all scientific reagents this product should be handled and stored with care as standard practice. Wear gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

Quick Start Protocol

Label PCR tubes with a fine permanent marker, and make a list of samples and tube numbers.

If using Ready-To-Use Primer Mixes

1. Make up a 1x Master Mix for all samples to be amplified, by using 4 µL of 5x Master Mix, 2 µL of the Primer Mix and 11 µL of PCR grade water for each sample. For example, for 10 samples this would consist of 40 µL of 5x Master Mix, 20 µL of Primer Mix and 110 µL of PCR grade water. Mix well.
2. Pipette 17 µL of the 1x Master Mix into each PCR tube.

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3. Using new pipette tips each time, pipette 3 µL of DNA extract per sample into each tube, close, place in thermocycler, and run the appropriate PCR programme.

If using other primers

1. Make up an aliquot of primers to 10 µM working concentration (if they are not already 10 µM), with PCR grade water. PCR grade water can include nuclease-free water, autoclaved molecular grade water, and DEPC-treated water.
2. Make up a 1x Master Mix with 4 µL of 5x Master Mix, 0.4 µL of each 10 µM primer (assuming a desired final concentration of 0.2 uM), and 12.2 µL of molecular grade water. For 10 samples this would consist of 40 µL of 5x Master Mix, 4 µL of each 10 µM primer, and 122 µL molecular grade water.
3. Pipette 17 µL of 1x Master Mix into each PCR tube.
4. Using new pipette tips each time, pipette 3 µL of DNA extract per sample into each tube, close, place in thermocycler, and run the appropriate PCR programme.

Table 1: Recommended PCR Cycle Conditions

Operation	Temp. °C	Time	No. cycles
Initial denaturation	95	3 – 5 min	1
Denaturation	95	30 sec	35
Annealing	54 – 66*	30 sec	
Extension	72	40 sec – 4 min**	
Final extension	72	5 – 10 min	1

**Annealing temperature depends on the primers and protocols used*
***Extension time should be ~1 min per 1,000 bp of PCR product*

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