# 5x HOT FIREPol® Blend Master Mix Ready To Load

Technical Data Sheet


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

*For research and educational use only.*

## Description

A PCR master mix is a 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.

HOT FIREPol® Blend Master Mix Ready to Load is an optimised ready-to-use PCR master mix for PCR assays requiring higher fidelity amplification and hot start functionality.

It contains a chemically deactivated FIREPol® DNA Polymerase that prevents the possibility of mispriming or primer dimer formation during room temperature setup. The polymerase is reactivated with a 12 – 15 min heating step as part of the initial denaturation step during PCR.

It also contains two carefully optimized enzymes: HOT FIREPol® DNA polymerase and a proofreading polymerase. The enzyme blend has 5’→ 3’ exonuclease activity and 3’→ 5’ proofreading activity for increased copy fidelity. This results in an increased fidelity (up to five fold) compared to regular Taq polymerase. Generated PCR products are compatible with blunt-end and T/A cloning procedures.

This master mix also contains dNTPs, $MgCl_2$, BSA, and buffer.

## Application Recommendations

### Ideal for routine and more demanding PCR applications

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

### Specifically designed for room-temperature setup

The hot start functionality prevents polymerase activity until the reaction mix is activated, reducing the chance of mispriming and primer dimer formation and improving specificity.

### Ideal for high-fidelity PCR applications

The optimised enzyme blend provides proof-reading functionality for applications where high-fidelity DNA amplification is a priority, such as those involving sequencing.

**Compatible with standard PCR instruments**

HOT FIREPol® Blend Master Mix Ready to Load is compatible with standard PCR instruments, and is a superior alternative to standard Taq Polymerase Master Mixes.

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

HOT FIREPol® Blend 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**

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

- **HOT FIREPol® DNA polymerase:** chemically modified FIREPol® DNA Polymerase enabling hot start
- **Proofreading enzyme**: to enhance copy fidelity – up to 5x that of standard Taq polymerases
- **5x Blend Master Mix Buffer with 7. 5 mM MgCl2**: 1x PCR solution – 1.5 mM MgCl 2
- **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**

## Storage & Stability

Routine storage at –20ºC. Temporary storage at room temperature while in use has no detrimental effects on the quality of this reagent.

Optionally aliquot into sterile tubes and freeze to minimise potential microbial contamination after opening.

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 HOT FIREPol® Blend 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.

1. Add 4 µL of 5x Master Mix, 2 µL of Bento Lab primer mix, and 10 µL of PCR grade water into each PCR tube.
2. Using new pipette tips each time, pipette 4 µL of DNA extract per sample into each tube, close, place in thermocycler, and run the appropriate PCR programme.

It is good practice to include a PCR tube that contains Master Mix, primer and PCR grade water but not DNA. This should be run on the gel alongside your samples to ensure there is no contamination of any of these reagents.

### Recommended PCR protocol

| Operation | Temp. °C | Time | No. cycles |
|---|---|---|---|
| Initial denaturation | 95 | 12 – 15 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