2X PCR HotStart Mastermix

Cat. No.	Product Description	Size
HTP01-01	2x HotStart PCR Mastermix, Blue	5x1 ml; 400 RXN
HTP01-02	2x HotStart PCR Mastermix, Blue	20x1 ml; 1600 RXN

Product Description

The 2xHotStart PCR Mastermix is a ready-to-use mixture of high quality HotStart DNA Polymerase, deoxynucleotides, reaction buffer, Mg^{2+}, a blue dye and stabilizing agent in a 2xconcentration. It contains all the necessary reagents for amplification of DNA. The HotStart Taq DNA polymerase is chemically modified by the addition of heat-labile blocking groups to its amino acid residues. The enzyme is inactive at room temperature and can only be activated after 10 minutes of incubation at 94°C, avoiding extension of non-specifically annealed primers or primer dimers and providing higher specificity of DNA amplification.

The blue dye included allows direct loading of the final products onto a gel for analysis, which resolves during gel electrophoresis into a blue band at 4kb.

To set up a PCR reaction: add DNA template, primers and water. PCR products that are amplified up to 6 kb in length with HotStart Taq DNA Polymerase contain a single base (A) 3' overhang, which can be cloned in TA vector.

Features and Benefits

- Saves preparation time by combining HotStart DNA Polymerase, dNTPs and reaction buffer in a ready-to-use mixture.
- Reduces the risk of contamination by decreasing the number of pipetting steps.
- Provides consistent reaction performance and results.

Storage

Keep at –20°C for long term storage. 2xHotStart PCR Mastermix is stable at 4°C for three months or for ten freeze-thaw cycles. For daily use, we recommend keeping an aliquot at 4°C.

Protocol

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross contamination. The use of clean filter tips is recommended. Always keep the control DNA and other templates to be amplified separately from the other components.

A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final Concentration
Template DNA	~50 ng	~1 ng/µl
Forward primer (10 µM)	0.5 µl	200 nM
Reverse primer (10 µM)	0.5 µl	200 nM
2xHotStart PCR Mastermix	12.5 µl	1x
Nuclease-free H_2O	up to 25 µl	

   - *We recommend preparing a Mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.*
2. Mix contents of tube and centrifuge briefly.
3. Incubate tube in a thermal cycler at 94°C for 10 mins to completely activate the HotStart DNA Polymerase and denature the template.
4. Perform 30 - 35 cycles of PCR amplification as follows:
   Denature: 94°C for 30 sec
   Anneal: 45 - 72°C for 30 sec
   Extend: 72°C for 1 min/1 kb template
5. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or Safe Gel Stain (Cat No. SAFE01-01) staining. Load the samples directly and Use appropriate molecular weight standards.

For laboratory research only. Not for clinical applications. For technical questions, please email us at service@bioland-sci.com or visit our website at www.bioland-sci.com