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2×HotStart HiFi PCR Master Mix with Dye

Catalog Number: D019-1, D019-2

Table 1. Contents and Storage

Content	D019-1	D019-2	Storage	Stability
2×HotStart HiFi PCR Master Mix	5×1 mL	10×1 mL	-20 °C, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.

Product Description

The 2×HotStart HiFi PCR Master Mix contains ABP HiFi DNA Polymerase, dNTP, Mg^{2+}, and an optimized buffer system. The stabilizer and enhancer in the master mix ensure the reagent stability and optimize the amplification reaction. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. HotStart HiFi PCR Master Mix contains inert, non-toxic dyes to visualize PCR mixing step, and allows direct loading of PCR products on to gels for electrophoresis.

The ABP HiFi DNA Polymerase is a high-fidelity DNA polymerase that combines both amplification efficiency and fidelity. It possesses 5'→3' polymerase activity and 3'→5' exonuclease activity, and the amplified product is blunt-ended, suitable for fragment amplification of the seamless cloning kit and amplification of the second-generation sequencing library. The ABP HiFi DNA Polymerase has an extension rate of 15-30 s/kb, and its fidelity is 50 times higher than that of conventional Taq, overcoming the shortcoming of low amplification efficiency and slow amplification speed of ordinary Pfu enzyme, thus shortening the reaction time.

This master mix has broad adaptability to PCR templates and strong resistance to inhibitors, and can be used for the amplification of DNA or cfDNA templates extracted from various samples such as FFPE, saliva, stool, urine, or blood.

Special Features

- Convenient, ready-to-use mix.
- Fast PCR.
- High-fidelity.
- Generates blunt-ended PCR products.

Applications

- High-fidelity PCR.

General Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of HotStart HiFi DNA polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized. Critical parameters and troubleshooting information are documented in reference 1.

Assemble PCR reactions in a nuclease-free environment. Use of “clean” dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.
2. Prepare the following reaction mixture in a PCR tube on ice:

Component	Volume	Final Concentration
Template DNA	x µL	1-500 ng
2×HotStart HiFi PCR Master Mix	25 µL	1×
Forward Primer (10 µM)	1 µL	200 nM
Reverse Primer (10 µM)	1 µL	200 nM
Nuclease-free H_2O	to 50 µL	-

3. Mix, and then briefly centrifuge the contents.
4. Perform PCR using the recommended thermal cycling conditions outlined below:

Steps	Temperature	Duration	Cycle
Initial Denaturation	95°C	30 sec	1
Denaturation	95°C	10 sec	25-35
Annealing*	56-64°C	10 sec	
Extension	72°C	15-20 sec/kb	
Final Extension	72°C	15-20 sec/kb	1
Holding	4°C	-	1

5. Analyze the amplification products by agarose gel electrophoresis.

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

• Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.

• Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.

• Wear fresh gloves for DNA purification and reaction set up.

• Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.

• Always perform “no template control” (NTC) reactions to check for contamination.

Reference

Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, eds. (1990) PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, CA.