Super HIFI PCR Master Mix

Cat. No.: P2111, P2112, P2113

Contents:

Component	P2111 (100 rxns)	P2112 (1000 rxns)	P2113 (5000 rxns)
2X Super HIFI PCR Master Mix	1 ml × 1	1 ml × 10	10 ml × 5
GC Enhancer	0.25 ml × 1	1 ml × 2	10 ml × 1

Store at -20°C

Description

Super HIFI PCR Master Mix is a PCR premix containing hotstart proofreading DNA polymerase and contains the various components (except primers and templates) required for PCR reactions. The chemically modified ultra-high fidelity DNA polymerase in this Master Mix is extremely specific, with 100-fold fidelity of Taq. It can be used for molecular cloning, first-generation sequencing, site-directed mutagenesis, NGS library construction and other PCR requiring high accuracy. This amplification reagent has undergone strict quality control and functional verification to ensure the stability and reproducibility of the reagent to the greatest extent. This product is suitable for multiple amplification and amplicon library construction with initial DNA template of 1-100 ng, and is compatible with DNA templates from different sample types: cell or tissue DNA, formalin-fixed paraffin embedded (FFPE) sample DNA, cell-free DNA(cfDNA), etc.

Applications

- High-specificity amplification & multiplex PCR
- High fidelity PCR

Basic PCR Protocol

Note: prepare Primer Mix with each Primer concentration of 0.5 μM before the experiment.

1. Add the following components to a sterile microcentrifuge tube sitting on ice (not necessary):

Reagent	Amount	Final Conc.

2X Super HIFI PCR Master Mix	10 μl	1X
Primer Mix (0.5μM each)	2 μl	50nM each primer[1]
Template DNA	40~80ng	2~4ng/μl
GC Enhancer	0/2.4 μl[2]	0/12%
Water, nuclease-free	to 20 μl	–

[1] Recommended range of final primer concentration: 0.05-0.4μM. For most reactions, a 0.15μM is able to give desirable results. 0.4 μM could increase the amount of product.
[2] GC Enhancer should be used only when target sequences with high GC content cannot be efficiently amplified.

2. Mix reagents in the tube. Cap tubes and centrifuge briefly to collect the mixture to the bottom.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	95°C	3 minutes
25-35 Cycles	95°C	30 seconds
	55-68°C[1]	90 seconds
	72°C	variable[2]
Final Extension	72°C	5-10 minutes

[1] The annealing temperature should be set according to the primer with lower Tm value.
[2] The optimal extension time should be set according to 2 kb /min.

4. Maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by nucleic acid dye staining. Use appropriate molecular weight standards.

Notes on cycling conditions

- This Master Mix adopts improved chemical modification technology, so it relies on temperature to activate the polymerase activity, which can effectively inhibit non-specific bindings.
- To avoid primer degradation due to enzyme correction activity, prepare the reaction system on ice.
- This Master Mix accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- dUTP, dITP and primers containing these nucleotides cannot be used for PCR amplification catalyzed by proofreading DNA polymerase. Because when the enzyme binds to a DNA template containing uracil or hypoxanthine, it stops the DNA polymerization process.

Guidelines for preventing contamination of PCR

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 use 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.

Quality Control

The absence of endodeoxyribonucleases, exodeoxyribonucleases and ribonucleases is confirmed by appropriate quality tests. Functionally tested in amplification of a single-copy gene from human genomic DNA.

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to a nicked DNA was observed after incubation of 25 µl Super HIFI PCR Master Mix (2X) with 1µg pBR322 DNA for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA-HindIII fragments was observed after incubation of 25 µl Super HIFI PCR Master Mix (2X) with 1µg digested DNA for 4 hours at 37°C and 70°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 25 µl Super HIFI PCR Master Mix (2X) with 1µg E.coli [3H]-RNA (40000cpm/µg) for 4 hours at 37°C and 70°C.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.