2 × FastHiFi PCR MasterMix

User's Instruction

Description

2 × FastHiFi PCR MasterMix is based on engineered Pfu DNA polymerase, which enables fast PCR with an extension time of 2-4kb/min. The mastermix generates blunt-end PCR products because of the 3' → 5' exonuclease (proofreading) activity, which shows approximately 55-fold higher fidelity than Taq DNA polymerase. Therefore, 2 × FastHiFi PCR MasterMix provides greater efficiency and elongation capabilities than conventional PCR enzymes. This mastermix contains red tracking dye, which can be directly loaded for electrophoresis without adding a loading buffer.

Kit Contents

2 × FastHiFi PCR MasterMix	5 ml

Features

- **Fast amplification speed:** The extension rate can reach 2-4 kb/min, which is 4-8 times that of Pfu DNA Polymerase.
- **High amplification yield:** Generally, the amount of PCR products is 50%-100% higher than that of traditional Pfu DNA Polymerase.
- **Excellent fidelity:** The fidelity is more than 55 times that of Taq DNA Polymerase.
- **Ideal for short sequences:** When using complex genomic DNA as a template, the amplification products shall be no more than 3kb. When using simple genomic, plasmid, and phage DNA as templates, the amplification products shall be no more than 6kb. For long sequence, we recommend 2× LongHiFi PCR MasterMix.

Quality Control

Each batch of 2 × FastHiFi PCR MasterMix must pass the amplification detection of the following two sizes of fragments before shipment:

- *Poly(A) Polymerase* gene from E. coli gDNA, 1.4 kb, extension 20 sec/kb
- *Hα1AT gene* from Human gDNA, 2.6 kb, extension 30 sec/kb.

Protocol

1. Set up the PCR reaction on ice as the following table:

Reagent	Volume	Final Concentration
2 × FastHiFi PCR MasterMix	25 μL	1 ×
Forward Primer (10 μM)	1 μL	0.2 μM
Reverse Primer (10 μM)	1 μL	0.2 μM
Template	X μL	-
ddH_2O	up to 50 μL	-

Recommended template amount:
Plasmid: 0.1-10ng. Bacterial genome: 10-100ng. Human genome: 50-150ng. cDNA: 1-5 μL from RT reaction.

2. Mix the reaction system (gently blow several times with a pipette to fully mix or gently mix with a vortex mixer at a lower speed), and then centrifuge to precipitate the liquid.

Thermocycling Conditions for PCR

Step	Temperature	Time	Cycle Number
Initial Denaturation	95°C	3 min	
Denaturation	95°C	10 sec	30 cycles
Annealing	55°C	10-15 sec	
Extension	72°C	15-30 sec/kb	
Final Extension	72°C	2-5 min	
	4-8°C	Hold	

Notes

- *Simple templates such as plasmid and simple genome can be extended at 15-20 sec/kb. Complex templates, such as the human genome, can be extended at 30-45 sec/kb.*
- *For templates with high GC content, the initial denaturation and denaturation temperature can be increased to 98°C, which will also not influence the activity of the mastermix.*
- *If the GC content of the amplification template is high or the template is complex and*

the amplification result is not good, DMSO can be added to the final concentration of 1%-8%. Increase the concentration of DMSO at the gradient of 1% to optimize. Or add betaine to the final concentration of 1.0-1.7 M and use Touchdown PCR.

Storage

The minimum shelf life is 2 years at -20°C or 1 month at 4°C.

Only for research and not intended for treatment of humans or animals