

2 × CYA9 LongHiFi PCR Master Mix

Product Description	Size	
	CYPC8401	CYPC8402
2×CYA9 LongHiFi PCR Master Mix	1 ml	5 ml

Store: -20°C, 2 years

Product Description

The kit contains CYA9 LongHiFi DNA Polymerase, dNTPs and optimized reaction buffer at a concentration of 2×. Before reaction, DNA/cDNA template, specific primer, and ddH₂O should be prepared to mix the final concentration of 1×.

CYA9 kits are the mixture of super-fidelity enzymes and a variety of elongation factors which enables CYA9 to greatly improve the amplification length, speed, fidelity and the yield. It is the better choice for the amplification of long fragments, high-speed and super-fidelity.

The Mix contains red tracer dye for quicker electrophoresis. And the PCR amplification products are with blunt end, which can be directly employed to the clone reaction with our pTOPO vector. Of course, after purified, the amplification products also can be used for other follow-up experiments, such as digestion, ligation and fluorescence sequencing.

Product Features

- High yield: 0.5–1 times that of Pfu;
- Fast amplification: 3-4kb/min;
- Super Fidelity: Up to 54 times of traditional Taq DNA polymerase;
- Powerful amplification capacity: suitable for the amplification of less than 10kb fragments (complicated genome DNA template) and less than 15kb sequences (simple gDNA, plasmid and so on);

Recommended PCR system settings:

Component	25 µl Reaction	50 µl Reaction	Final Concentration
2× CYA9 PCR MasterMix	12.5 µl	25 µl	1 ×
Forward Primer (10 µM)	0.5 µl	1 µl	0.2 µM
Reverse Primer (10 µM)	0.5 µl	1 µl	0.2 µM
Template DNA	as required	as required	
ddH ₂ O	up to 25 µl	up to 50 µl	

Reference template dosage (50 µl reaction system)

Plasmid: 0.1-10ng; Bacterial genome: 10-100ng; Human genome: 50-150ng;

cDNA: 1-5µl from RT reaction;

Proposed PCR cycle conditions

Step	Temperature	Time	Cycle Number
Initial denaturation	95°C	3 minutes	
Denaturation	95°C	10 seconds	30 cycles
Annealing	55°C	10-15 seconds	
Extension	72°C	15-20 seconds / kb	
Final Extension	72°C	2-5 minutes	
	4-8°C	Hold	

Precautions:

1. Due to CYA9 fast amplification capacity, 10-15s/kb extension time is recommended for simple or plasmid templates; 20s-25s/kb extension time for complicated template;
2. If smearing above and below the main band is observed during electrophoresis, the extension time is too long. Decreasing the extension time is recommended.
3. For templates with high GC content, the pre-denaturation and denaturation temperatures can be increased to 98°C, a temperature which has no clear influence on CYA9 activity.
4. If the amplification template GC content is high or the complex template amplification effect is poor, the DMSO can be added to the reaction mixture with the final concentration of 1-8%, according to 1% gradient increasing to look for the best concentration. Or adding betaine to the final concentration of 1.0-1.7 M. And at the same time, using the Touchdown PCR.

Example Amplification

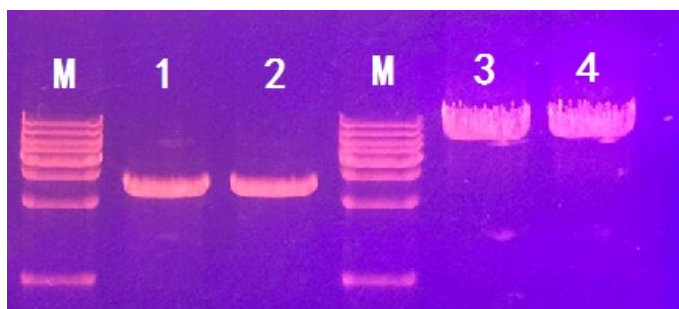


Figure 1. Amplification of DNA fragment from 50 ng human genomic DNA in 50 µl reaction mixture.

Lane M: 1kb ladder;

Lane 1-2: Hx1AT, 2.6Kb, extension 20 sec/kb;

Lane 3-4: β-globin, 6kb, extension 20 sec/kb.

This product is furnished for LABORATORY RESEARCH USE ONLY.

Not for diagnostic or therapeutic use.