2 × CYA8 FastHiFi PCR Master Mix

Product Description	Size	
2×CYA8 FastHiFi PCR Master Mix	CYPC8201	CYPC8202
	1ml	5ml

Store: −20°C, 2 years

Product Description

The kit contains CYA8 FastHiFi DNA Polymerase, dNTPs and optimized reaction buffer at a concentration of 2x. Before reaction, DNA/cDNA template, specific primer, and ddH2O should be prepared to mix the final concentration of 1×.

A8 is from the genetic engineering modification Pfu, which can greatly improve the amplification rate, fidelity and yield. So A8 Mix is the preferred product for non-long fragments ultra-fidelity fast amplification.

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 experiment, such as digestion, ligation and fluorescence sequencing.

Product Features

1.Fast amplification: 2-4kb/min, up to 4-8 times of Pfu;
2.High Yield: Up to 1.5-2 times of traditional Pfu;
3.Super Fidelity: Up to 54 times of traditional Taq;
4.With complex genomic DNA as template for the amplification of not more than 3kb of the product, with simple genome, plasmid and phage DNA as a template for the amplification of not more than 6kb of the product.

Recommended PCR system settings:

Component	25 μl Reaction	50 μl Reaction	Final Concentration
2× A8 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_2O	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	15-30 seconds / kb	
Final Extension	72°C	2-5 minutes	
	4-8°C	Hold	

Precautions:

1. CYA8 amplification for plasmid or other simple genome: 15-20s/kb; for complex genome such as human genome: 30-45s/kb;

2. For templates with high GC content, the pre-denaturation and denaturation temperatures can be increased to 98 oC; A8 is heat resistance, and A8's activity will not be influenced under the 98 oC environment;

3. 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 1.0-1.7M. And at the same time, using the Touchdown PCR.

Example Amplification

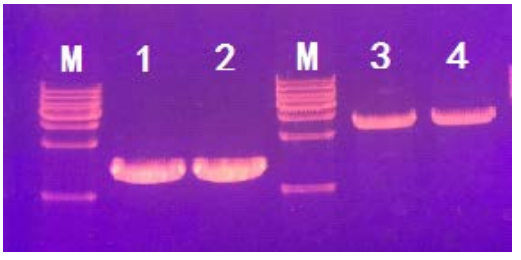

Figure.1. Amplification of DNA fragment from 50 ng genomic DNA in 50μl reaction mixture.
Lane M: 1kb ladder;
Lane 1-2: Poly(A) Polymerase from E. coli gDNA, 1.4Kb, extension 20 sec/kb;
Lane3-4: Hα1AT from Human gDNA, 2.6Kb, extension 30 sec/kb.

This product is furnished for LABORATORY RESEARCH USE ONLY.
Not for diagnostic or therapeutic use.