Ver.EN20240517

2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix

Product description

2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix contains heat-stabilized Taq DNA Polymerase modified with antibodies, adds strong elongation factor and optimized buffer system, and has super high amplification efficiency. It is very suitable for PCR amplification of most colonies such as *E. coli*, *Agrobacterium*, and yeast. The extension speed of amplification of complex templates within 3 kb can reach 1 sec/kb, 3-6 kb to 3 sec/kb, 6-10 kb to 5 sec/kb and 10 kb to 10 sec/kb; the amplification speed of simple templates such as plasmid within 6 kb can reach 1 sec/kb, which can greatly save PCR reaction time. Meanwhile, Mix contains dNTPs, Mg^{2+}, and it can be amplified only with adding primers and template. In addition, Mix contains red tracer dye, which can be used by electrophoresis directly after the end of the reaction. The protective agent added to the system enables the product to maintain a stable activity after repeated freezing and thawing. The 3′ end of the PCR product bands A and can be easily cloned into the T vector.

Components

Name	10167ES03	10167ES08	10167ES50	10167ES60
2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix	1 mL	5×1 mL	50×1 mL	100×1 mL

Specifications

Product specification	Master Mix
Concentration	2×
Hot Start	Built-in Hot Start
Overhang	3 '-A
Reaction speed	Rapid
Size (Final Product)	Up to 15 kb
Conditions for transportation	Dry ice

Storage

The 2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix products should be stored at -25~-15°C for 2 years.

Instructions

1. Reaction System

Components	Size (μL)	Size (μL)	Final concentration
2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix*	25	12.5	1×
Template DNA**	suitable	suitable	-
Forward primer (10 μmol/L)***	2	1	0.4-0.5 μM
Reverse primer (10 μmol/L)	2	1	0.4-0.5 μM
ddH_2O	to 50	to 25	-

Table 1 Reaction system

*The 1× Mix contains 2 mM Mg^{2+} and 200 μM dNTPs, thawed thoroughly before use.

**_E. coli_ and _Agrobacterium_ can directly absorb bacterial liquid or pick bacteria samples; It is recommended that the yeast liquid and strain be boiled for 5 min, then put into the -80°C refrigerator for 3 min, and then serve the sample after thawing. Note that the bacterial liquid sample should be shaken and mixed before sampling, in which the recommended amount of bacterial liquid sample is 2-4 μL (0.5-0.8 OD_{600}).

Recommended usage of the different templates:

Type of template	Segment usage range (25 μL reaction system)
Genomic DNA	10–1,000 ng
Plasmid or λDNA	0.5-50 ng
E. coli bacteria solution	0.5-0.8 OD_{600}

Table 2 Recommended usage of different templates

***The range of final primer concentration in PCR reaction system is 0.2-1 μM, and 0.4 μM is recommended.

2. Amplification Protocol

Cycle steps	Temperature (°C)	Time	Cycles
Predenaturation	95	3 min	1
Denaturation	95	15 sec	30-35
Annealing*	60	20 sec	
Extension**	72	1-10 sec/kb	
Final extension	72	5 min	1

Table 3 Amplification protocol

*Recommended annealing temperature: 60°C, you can also set up a temperature gradient according to your own needs to explore the optimal temperature for primer annealing. The recommended annealing time is set to 20 sec, which can be adjusted within 10-30 sec. Too long annealing time may lead to dispersion of the amplified product on the glue.

**Extension speed: 1 sec/kb for complex templates such as genomes and _E. coli_ within 3 kb, 3 sec/kb for complex templates within 6 kb, 5 sec/kb for most complex templates within 10 kb, and 10 sec/kb for complex template fragments over 10 kb. For simple templates, such as plasmids less than 6 kb, set 1 sec/kb; for simple templates, such as 6-10 kb plasmids, set 3 sec/kb; and for simple templates, such as plasmids larger than 10 kb, set 5-10 sec/kb. If it is necessary to increase production, the extension time can be extended appropriately, and should not exceed 30 sec/kb.

Notes

1. For your safety and health, please wear lab coats and disposable gloves for operation.
2. This product is for research use ONLY!