2 × KeyPo SE Master Mix (Dye Plus)

PK512

Version 24.1

Product Description

KeyPo SE DNA Polymerase is a new generation of high-fidelity DNA polymerase based on the BioSmart platform, which features an ultra-high amplification success rate and rapid amplification of 15 sec/kb. With the latest hot start technology and optimized buffer system, it can perform highly specific singleplex PCR for up to 40 kb, and highly uniform multiplex PCR within the range of 300 bp - 15 kb, which minimizes GC bias and allows for more uniform amplification of various regions of genomic DNA and cDNA. 2 × KeyPo SE Master Mix (Dye Plus) is a premix containing KeyPo SE DNA Polymerase, dNTPs, Mg^{2+}, and other necessary components for PCR reactions. It only requires the addition of primers and templates for amplification, thereby reducing pipetting operations and improving detection throughput and reproducibility of results. It is applicable for regular templates, crude samples, uracil-containing DNA, long fragments (40 kb), and GC-rich systems (primers or templates). This product contains tracking dyes, and amplification products can be directly loaded for gel electrophoresis after the reaction. The amplification products are blunt-ended, which are subsequently applicable for ClonExpress Ultra One Step Cloning Kit V2 (Vazyme #C116) and Ultra-Universal TOPO Cloning Kit (Vazyme #C603).

Components

Components	PK512-01	PK512-02	PK512-03
2 × KeyPo SE Master Mix (Dye Plus)	1 ml	5 × 1 ml	15 × 1 ml

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

It is applicable for PCR using genomic DNA, cDNA, plasmids, uracil-containing DNA, and crude samples as templates.

Notes

1. Please use high-quality templates to increase the success rate and yield.
2. KeyPo SE DNA Polymerase has the strong proofreading activity. If TA cloning needs to be performed, it is recommended to purify the DNA before adding the adenine.
3. Primer Design Guidance:
   a. It is recommended that the last base at the 3' end of the primer should be G or C.
   b. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer, while also avoiding hairpin structures.
   c. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1°C. The Tm value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the Tm value).
   d. The Tm difference between the primers in multiplex PCR should be as small as possible, while minimizing the complementary pairing between the primers.
   e. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value. The recommended GC content of primers is 40% - 60%.
   f. The overall distribution of A, G, C, and T in the primer should be as even as possible. Avoid using regions with high GC or AT contents.
   g. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers and avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
   h. When amplifying fragments (≥5 kb), the length of the primer should be 25 - 35 nt and the Tm value should be >65°C.
   i. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

Experiment Process

1. Reaction System

Perform all operations on the ice during the experiment. Thaw, mix, and briefly centrifuge each component before use. After use, please return it to -20°C in time for storage.

Components	Volume
ddH_2O	up to 50 μl
2 × KeyPo SE Master Mix (Dye Plus)	25 μl
Forward primer (10 μM)[a]	1.5 μl
Reverse primer (10 μM)[a]	1.5 μl
Template DNA[b]	x μl

a. For performing multiplex PCR: 1. The final concentration of each primer is 0.3 μM; If nonspecific products or smeared bands occur, please reduce the concentration of each primer to 0.1 μM; 2. The bias during multiplex PCR amplification can be improved by appropriately increasing the primer concentration of relatively weaker bands or reducing the primer concentration of over-amplified products; 3. Please perform singleplex PCR first, ensuring each primer pair can be successfully amplified.

b. Optimal reaction concentration varies in different templates. In a 50 μl system, the recommended template usage is as follows:

Template Types	Amount
Genomic DNA	10 - 200 ng
Plasmid or Virus DNA	10 pg - 50 ng
Uracil-containing DNA	50 - 500 ng
cDNA	1 - 5 μl (≤1/10 of the total volume of PCR system)
Crude samples	1 - 5 μl (≤1/10 of the total volume of PCR system)

2. Reaction Program

◇ Standard program

Steps	Temperature	Time	Cycles
Initial Denaturation	94°C	2 min	
Denaturation	98°C	10 sec	28 - 35
Annealing[a]	Tm	30 sec	
Extension[b]	68°C	15 - 30 sec/kb	

a. Please set the annealing temperature according to the Tm value of the primers. When the Tm value of the primers is higher than 68°C, the annealing step can be removed (Two-Step PCR). If necessary, the annealing temperature can be further optimized through setting the temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Increasing the annealing temperature can improve the specificity of amplification.

b. Please refer to the following table to set the extension time based on the length of the target fragment:

PCR Types	Size	Recommended Extension Time
Singleplex PCR	<10 kb	15 sec/kb
	≥10 kb	30 sec/kb
Multiplex PCR	<10 kb (longest fragment)	30 sec/kb
	≥10 kb (longest fragment)	60 sec/kb
Direct PCR (Templates such as plant leaves, blood, and cells were not lysed for PCR)	/	30 sec/kb
Uracil-containing PCR	/	30 sec/kb

◇ Stepdown program

Temperature	Time	Cycles
94°C	2 min	
98°C	10 sec	5
74°C	30 sec/kb	
98°C	10 sec	5
72°C	30 sec/kb	
98°C	10 sec	5
70°C	30 sec/kb	
98°C	10 sec	15 - 25
68°C	30 sec/kb	
68°C	7 min	

▲ It is applicable for amplification failure or poor specificity of long fragments system [>10 kb] using standard program.