2× S705 HotPrime Mix

REF: EG26104-S/M/L

Storage Condition

Store at -20℃ for 2 years.

Components

Component	EG26104S	EG26104M	EG26104L
2× S705 HotPrime Mix	1 ml	5×1 ml	20×1 ml

Description

2× S705 HotPrime Mix is a ready-to-use 2× premixed solution containing S705 HotPrime DNA Polymerase, dNTPs, and an optimized reaction buffer. PCR with high fidelity can be performed simply by adding template, primers, and water. S705 HotPrime DNA Polymerase is antibodyblocked and fully inhibited at room temperature. Full activity is restored after heating at 95℃ for 30 s, minimizing nonspecific amplification.

Application

Suitable for PCR amplification using genomic DNA, cDNA, plasmid DNA, or crude samples as templates.

Protocol

1. Reaction System

Reagent	Amount
2× S705 HotPrime Mix [a]	25 μl
Forward primer (10 μM)	1 μl
Forward primer (10 μM)	1 μl
Template DNA[c]	x μl
ddH_2O	Up to 50 μl

a. When amplifying fragments with GC content >60% and standard conditions fail, Touchdown PCR is recommended.

2. Recommended template amounts and reaction System

(1) Template amount:

Templates types	Recommended amount
Genomic DNA	10~200 ng
Plasmid or viral DNA	10 pg~50 ng
cDNA*	1~20 μl
Crude template	1~5 μl (≤ 1/10 of total PCR volume)

Note: The recommended cDNA volume is for BestEnzymes Biotech reverse transcription products. Adjust volume if using other reverse transcription kits to avoid PCR inhibition.

(2)Program:

① Three-step:

Step	Temperature	Time	
Pre-denaturation[b]	95℃	3~5 min	
Denaturation	95℃	10 s	30~35 Cycles
Annealing[c]	55~72℃	15 s	30~35 Cycles
Extension[d]	72℃	30 s/kb	30~35 Cycles
Final Extension	72℃	5 min	

b. An initial denaturation of 30~60 s is sufficient for most templates; for complex templates, such as GC-rich sequences, it is recommended to extend the initial denaturation time to 3~5 minutes. E. coli colony PCR: It is recommended to adjust the pre-denaturation time to 10 min, enabling efficient amplification without pre-lysis. Yeast colony PCR: No need to adjust the pre-denaturation time, but prelysis of yeast is required. Recommended lysis methods: Add NaOH to yeast to a final concentration of 20 mM, then pre-lyse at 95℃ for 10 min; or repeatedly freeze thaw yeast 2~5 times using liquid nitrogen.

c. Set the annealing temperature according to the Tm value of the primers. If the Tm value of the primers is ≥72℃ , the annealing step can be omitted, allowing the protocol to proceed directly to the extension step (two-step PCR). If necessary, A temperature gradient can also be used to optimize the annealing temperature for each primer pair. Additionally, the annealing temperature has a direct impact on the specificity of amplification. If poor amplification specificity is observed, it may be beneficial to increase the annealing temperature accordingly.

d. For most templates, an extension time of 30 s/kb is adequate for effective amplification; However, for some complex templates, the extension time may be extended to 30~60 s/kb.

② Two-step[e]:

Step	Temperature	Time	
Pre-denaturation	95℃	3~5 min	
Denaturation	95℃	10 s	30~35 Cycles
Annealing & Extension	65~68℃	30 s/kb	30~35 Cycles
Final Extension	72℃	5 min	

e. In general, there is no significant difference between the two-step and three step programs for PCR amplification. However, for some complex templates (such as long template, uneven Tm distribution, or templates with special structures), it is recommended to the two-step program or Touchdown PCR. Furthermore, when using plasmids as templates for site-directed mutagenesis, the two-step program is also recommended.

③ Touchdown PCR:

Step	Temperature	Time	
Pre-denaturation	95℃	3~5 min	
Denaturation	95℃	10 s	30~40 Cycles
Annealing	68℃ (-0.2℃ /cycle)	15 s	30~40 Cycles
Extension	72℃	30 s/kb	30~40 Cycles
Final Extension	72℃	5 min	

f. This program is for reference only. Touchdown PCR offers various protocols that can be flexibly adjusted according to the experimental requirements.

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Notice

1. Please do not use primers and templates containing uracil, or dUTP.

2. S705 High-Fidelity DNA Polymerase has strong proof-reading activity, and its amplification products have blunt ends. If T/A-cloning is the next step, the amplification products should be purified prior to A-addition.

3. To improve the success rate and yield of amplification, please use high-quality templates.

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