2×Taq-AS PCR Mix

REF: EG21103-S/M

Storage Condition

-20℃

Components

Component	EG21103S	EG21103M
2×Taq-AS PCR Mix	1 ml	5×1 ml

Description

Taq-AS PCR Mix is a ready-to-use PCR mix with the concentration of 2×. It offers convenience and reduces the risk of contamination. PCR products synthesized with this mix have an overhang of A bases at the 3' end, enabling direct T/A cloning after purification.

The Taq DNA polymerase in this mix is a mutated variant called Taq-AS (Advanced Strong) DNA Polymerase, which is able to amplify DNA fragments up to 5 kb efficiently. It exhibits excellent tolerance to inhibitors and has a three-fold faster amplification rate compared to wild type Taq DNA polymerase, significantly reducing the time required for PCR extension. Unlike fusion protein-based fastTaq polymerases, Taq-AS behaves more similarly to WT-Taq, minimizing issues such as band smearing, trailing, or alteration of fragment sizes.

Quality Control Assays

Endonuclease Activity

A 50 μl reaction containing 200 ng of supercoiled plasmid and 25 μl of 2×Taq-AS PCR Mix incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 50 μl reaction containing 15 ng of dsDNA fragments and 25 μl of 2×Taq-AS PCR Mix incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

1. PCR Reaction System

Reagent	Amount	Final Concentration
2×Taq-AS PCR Mix [a]	25 μl	1×
Forward Primer (10 μM)[b]	1~2 μl	0.2~0.4 μM
Reverse Primer (10 μM)[b]	1~2 μl	0.2~0.4 μM
Template DNA[c]	x μl	
ddH_2O	To 50 μl	

a. Thaw out before use.

b. Recommended final primer concentration is 0.2~0.4 μM. Adjustments can be made in the range of 0.1~1 μM.

c. Optimal reaction concentrations may vary for different templates. For a 50 μl reaction system, the recommended template amounts are approximately 10~400 ng for genomic DNA and 10 pg~20 ng for plasmid or viral DNA.

2. Three-Step PCR Program

Step	Temperature	Time	
Initial Denaturation[d]	94℃	3~5 min	
Denaturation	94℃	30 s	30~35 Cycles
Annealing	55~65℃	30 s	
Extension	72℃	20~40 s/kb	
Final Extension	72℃	5 min	

3. Two-step PCR Program (for Target Fragments ≥3 kb)

Step	Temperature	Time	
Initial Denaturation [d]	94℃	3~5 min	
Denaturation	94℃	30 s	30~35 Cycles
Annealing and Extension	68℃	30 s/kb	
Final Extension	72℃	5 min	

d. For colony PCR, a longer initial denaturation (≥5 min) is beneficial for cell lysis.

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