2×Taq-AS PCR Mix (+Dye)

REF: EG21102-S/M/L

Storage Condition

-20℃

Components

Component	EG21102S	EG21102M	EG21102L
2×Taq-AS PCR Mix (+Dye)	1 ml	5×1 ml	100×1 ml

Description

Taq-AS PCR Mix (+Dye) 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.

The PCR mix contains two dyes, after reaction the sample could be loaded directly for gel electrophoresis. The dyes do not affect PCR amplification efficiency. However, for experiments requiring optical analysis such as absorbance or fluorescence of PCR products, it is recommended to purify the PCR product before analysis.

Quality Control Assays

Endonuclease Activity

A 50 μl reaction containing 200 ng of supercoiled plasmid and 25 μl of 2×Taq-AS PCR Mix (+Dye) 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 (+Dye) 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 (+Dye)[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.

BestEnzymes Biotech Co., Ltd.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com