Taq-Plus PCR Forest Mix (2×) V2

REF: EG25105-M/L

Storage Condition

-20℃

Components

Component	EG25105M	EG25105L
Taq-Plus PCR Forest Mix (2×) V2	5×1 ml	100×1 ml

Description

Taq-Plus PCR Forest Mix (2×) V2 is a pre-mixed PCR reaction solution formulated with Taq DNA Polymerase, dNTPs, and all of the components required for PCR, except DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to the fewer pipetting steps required for PCR set up.

It is capable of robust amplification of up to 6 kb from genomic DNA. PCR products amplified with this mix have a 3'-terminal adenosine (A), and therefore PCR products can be used directly for T/A cloning.

The Forest mix is supplemented with two tracking dyes that allows for direct loading of the PCR product on a gel. The dyes in the mix do not interfere with PCR performance but need to purify the PCR product when it's used for absorbance, fluorescence, etc. In addition, the dye-free Taq-Plus PCR Master Mix (2×) V2 can also be used.

Quality Control Assays

Endonuclease Activity

A 50 µl reaction containing 200 ng of supercoiled plasmid and 25 µl of Taq-Plus PCR Forest Mix V2 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 Taq-Plus PCR Forest Mix V2 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
Taq-Plus PCR Forest Mix (2×) V2	25 µl	1×
Forward Primer (10 µM)[a]	1~2 µl	0.2~0.4 µM
Reverse Primer (10 µM)[a]	1~2 µl	0.2~0.4 µM
Template DNA[b]	x µl	
ddH_2O	To 50 µl	

a. Recommended final concentration for primers is 0.2~0.4 µM. Adjustments can be made in the range of 0.1~1 µM.
b. 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. PCR Conditions

Step	Temperature	Time
Initial denaturation[a]	95℃	3~5 min
Denaturation	95℃	30 s
Annealing	55~65℃	30 s
Extension[b]	72℃	30~60 s/kb
Final Extension	72℃	5 min

30~35 Cycles

a. The initial denaturation condition is suitable for most amplification reactions. 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 pre-lysis 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.
b. It is recommended to set at 30 s/kb when the target fragment is shorter than 3 kb. If the length of the target fragment exceeds 3 kb, it is recommended to set at 60 s/kb.

3.Migration Distance of Dyes Corresponding to Gel Concentration

Agarose Gel Concentration	Orange Band	Blue Band
0.8%	~80 bp	4000 bp
1.0%	~40 bp	2000 bp
1.5%	~20 bp	1500 bp
2.0%	<10 bp	1200 bp
2.5%	<10 bp	1200 bp
3.0%	<10 bp	1200 bp

Note: The dyes can affect absorbance.

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