Taq-Plus PCR Forest Mix (2×)

REF: EG15139-M/L

Storage Condition

-20℃

Components

Component	EG15139M	EG15139L
Taq-Plus PCR Forest Mix (2×)	5×1 ml	100×1 ml

Description

Taq-Plus PCR Forest Mix (2×) 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×) 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 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 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×)	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]	94℃	3~5 min
Denaturation	94℃	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. For some complex templates, such as bacteria and yeast colon, the initial denaturation time can be extended to 10 minutes to improve denaturation efficiency.
b. It is recommended to set at 30 s/kb when the target fragment is shorter than 2 kb. If the length of the target fragment exceeds 2 kb, it is recommended to set at 60 s/kb.

Note: If yeast cell is used as the PCR template, it is recommended that the target fragment length does not exceed 2.5 kb. If it exceeds 2.5 kb, it is advisable to use the lysed cells as template.

3.Migration Distance of Dyes Corresponding to Gel Concentration

Agarose Gel Concentration	Gold 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.

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