

2×*TransStart*[®] *FastPfu* PCR SuperMix

Please read the datasheet carefully prior to use.

Cat. No. AS221

Version No. Version 4.0

Storage: at-18°C or below for two years

Description

TransStart[®] *FastPfu* PCR SuperMix is a ready-to-use mixture of *TransStart*[®] *FastPfu* DNA polymerase, dNTPs, and optimized buffer, featuring high amplification efficiency, fast amplification speed, high fidelity and high specificity. The SuperMix is provided at 2× concentration and can be used at 1× concentration by adding template, primers and H₂O for amplification. The amplification products are blunt-ended. The amplification products of 2× *TransStart*[®] *FastPfu* PCR SuperMix (-dye) can be directly cloned into *pEASY*[®]-Blunt series vectors. The amplified product of 2×*TransStart*[®] *FastPfu* PCR SuperMix (+dye) can be analyzed by electrophoresis directly, and need to be purified to remove dye when applied in cloning.

Its PCR product is not suitable for polyacrylamide gel electrophoresis.

- Reduce PCR operation time.
- Avoid contamination caused by the multi-step operation.
- *TransStart*[®] *FastPfu* PCR SuperMix offers 54-fold fidelity as compared to *EasyTaq*[®] DNA Polymerase.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Features

Fast, high fidelity, high specificity, good stability

Applications

- High fidelity PCR
- Site-directed mutagenesis
- Blunt end cloning
- Complex templates
- GC/AT-rich templates
- Long fragment amplification

Kit Contents

Component	AS221-01/11	AS221-02/12
2× <i>TransStart</i> [®] <i>FastPfu</i> PCR SuperMix (-dye)/(+dye)	1 ml	5×1 ml
Nuclease-free Water	1 ml	5 ml

Recommended PCR system and conditions (taking 50 µl reaction system as an example)

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 µM)	2 µl	0.4 µM*
Reverse Primer (10 µM)	2 µl	0.4 µM*
2× <i>TransStart</i> [®] <i>FastPfu</i> PCR SuperMix	25 µl	1×
Nuclease-free Water	Variable	-
Total volume	50 µl	-

The recommended final primer concentration is 0.4 µM; it can be adjusted within the range of 0.2-0.4 µM to optimize amplification specificity and yield.



Optimized parameters (50 µl reaction volumes)

Template	Input
Genomic DNA	10-500 ng
Plasmid DNA	1-30 ng
cDNA	1-2 µl cDNA from RT reaction (50-500 ng RNA for RT reaction)

PCR

Number of Cycles	Temperature	Time
1 cycle	98°C	1 min
30-35 cycles	98°C	10 sec
	Tm-5°C*	5 sec
	72°C	4 kb/min**
1 cycle	72°C	5 min

*The recommended annealing temperature is Tm-5°C; it can be adjusted within the range of Tm-5°C to Tm+5°C to optimize specificity.

**To enhance amplification efficiency and specificity of the product, the extension rate can be reduced to 2-4 kb/min for optimization.

Note

- Thoroughly thaw and mix when using.

For research use only, not for clinical diagnosis

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