TransStart® FastPfu Fly DNA Polymerase

Cat. No. AP231
Concentration: 2.5 units/μl
Storage: at -20°C for two years

Description

TransStart® FastPfu Fly DNA Polymerase is a hot start, high fidelity and high processivity DNA Polymerase. *TransStart® FastPfu* Fly DNA Polymerase has an xtension rate of up to 6 kb/min. Compared with *TransStart® FastPfu* DNA Polymerase, *TransStart® FastPfu* Fly DNA Polymerase has higher extension rate, higher fidelity, and higher amplification efficiency.

Highlights

- *TransStart® FastPfu* Fly DNA Polymerase offers 108-fold fidelity as compared to *EasyTaq®* DNA Polymerase.
- Extension rate is about 2-6 kb/min.
- PCR products can be directly cloned into *pEASY®*-Blunt vectors.
- Amplification of genomic DNA fragment up to 15 kb.
- Amplification of plasmid DNA fragment up to 20 kb.

Applications

- High fidelity PCR
- High yield and fast PCR
- Blunt end cloning
- Site-directed mutagenesis
- Complex templates

Unit Definition

One unit of *TransStart® FastPfu* Fly DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

TransStart® FastPfu Fly DNA Polymerase has passed the following quality control assays: functional absence of double- and single-strand endonuclease activity; >99% homogeneous measured by SDS-PAGE. Each batch of *TransStart® FastPfu* Fly DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

Storage Buffer

50 mM Tris-HCl (pH 8.2), 0.1 mM EDTA, 1 mM DTT, Stabilizers, 50% glycerol

5×*TransStart® FastPfu* Fly Buffer

100 mM Tris-SO_4 pH 9.2, 50 mM $(NH_4)_2SO_4$, 200 mM KCl; 10 mM $MgSO_4$; 10% Glycerol; others

Kit Contents

Component	AP231-01/11	AP231-02/12	AP231-03/13
TransStart® FastPfu Fly DNA Polymerase	250 U×1	500 U×1	500 U×6
5×*TransStart® FastPfu* Fly Buffer	1.2 ml×1	1.2 ml ×2	1.2 ml ×12
2.5 mM dNTPs	- / 500 μl×1	- / 1 ml ×1	- / 1 ml ×6
50 mM $MgSO_4$	200 μl×1	400 μl ×1	1 ml ×1
PCR Stimulant	200 μl×1	400 μl×1	1 ml ×1
6×DNA Loading Buffer	500 μl×1	1 ml ×1	1 ml ×2

PCR Stimulant

For better amplification of GC rich or complex template, we recommend adding PCR Stimulant into PCR reaction. PCR Stimulant is provided at 5× concentration and can be used at 0.5×-2.5× concentration.

Reaction Components

Component	Volume	Final Concentration
Template	Variable	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
5×*TransStart® FastPfu* Fly Buffer	10 μl	1×
2.5 mM dNTPs	4 μl	0.2 mM
TransStart® FastPfu Fly DNA Polymerase	1 μl	2.5 units
ddH_2O	Variable	-
Total volume	50 μl	-

Suggested conditions（50 μl reaction volume）

Parameter	Targets≤10 kb	Targets≥10 kb	cDNA Targets
Template	100 ng Genomic DNA 5-30 ng Plasmid DNA	200-500 ng Genomic DNA 5-30 ng Plasmid DNA	1-2 μl cDNA from RT reaction (50-500 ng RNA for RT reaction)
$MgSO_4$	Add 1-2 μl of 50 mM $MgSO_4$ to a final concentration of 3-4 mM for target larger than 5 kb		

Thermal cycling conditions

Number of cycles	Temperature	cDNA or Genomic DNA	Plasmid DNA
1 cycle	95°C	2 min	2 min
Plasmid or Genomic DNA: 30-35 cycles cDNA: 35-40 cycles	95°C	20 sec	20 sec
	Tm-5°C	20 sec	20 sec
	72°C	6 kb/min for targets≤2 kb 2-4 kb/min for targets>2 kb	6 kb/min for targets≤6 kb 2-4 kb/min for targets>6 kb
1 cycle	72°C	5 min	5 min

Notes

- For GC rich templates, the recommended denaturation temperature is 98°C.
- To ensure high fidelity, we recommended using high quality dNTPs. dNTPs containing dUTP cannot be used.

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