TransStart® *TopTaq* DNA Polymerase

Cat. No. AP151
Concentration 2.5 units/μl
Storage at -20°C for two years

Description

TransStart® *TopTaq* DNA Polymerase is an engineered version of *Taq* DNA Polymerase combined with *TransStart*® technique. One binding protein binds to double-strand DNA template, preventing polymerase activity at room temperature. Other two binding proteins bind primers, preventing primer-dimer formation. Blocking proteins are released from primers and templates during the initial denaturation. This double blocking method has higher efficiency than antibody based, or chemically modified hot start PCR.

Highlights

- Compared with *TransStart*® *Taq* DNA Polymerase, *TransStart*® *TopTaq* DNA Polymerase has higher amplification efficiency, specificity and sensitivity.
- *TransStart*® *TopTaq* DNA Polymerase offers 18-fold fidelity as compared to *EasyTaq*® DNA Polymerase.
- The specificity is higher than antibody based or chemically modified hot start DNA polymerases.
- Template-independent "A" can be generated at the 3' end of the PCR product. PCR products can be directly cloned into *pEASY*®-T vectors.
- Reduced nonspecific amplification and primer dimer formation.
- Different from *Taq* antibody, no risk of contamination from mammalian DNA.
- Different from chemical modification, long denaturing step is not needed.
- Amplification of genomic DNA fragment up to 15 kb.

Applications

- Complex templates
- GC/AT-rich templates
- Multiplex PCR
- High yield PCR

Unit Definition

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

Quality Control

TransStart® *TopTaq* 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*® *TopTaq* DNA Polymerase has been assayed for amplification efficiency to amplify p53 gene from 10 ng of human genomic DNA.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 50% glycerol, stabilizers

10×*TransStart*® *TopTaq* Buffer with 20 mM $MgSO_4$

500 mM Tris-HCl (pH 9.0), 200 mM $(NH_4)_2$ SO_4, 20 mM $MgSO_4$, others

GC Enhancer

For better amplification of GC rich or complex templates, we recommend adding GC enhancer to PCR reaction. GC enhancer is provided at 10× concentration and can be used at 0.5×-5× concentration.

Kit Contents

Component	AP151-01/11	AP151-02/12	AP151-03/13
TransStart® TopTaq DNA Polymerase	250 U×1	500 U×1	500 U×6
10×*TransStart® TopTaq* Buffer	1.2 ml	1.2 ml×2	1.2 ml×12
2.5 mM dNTPs	-/800 µl×1	-/800 µl×2	-/1.2 ml×8
10×GC Enhancer	200 µl×1	400 µl×1	1 ml×1
6×DNA Loading Buffer	500 µl×1	1 ml×1	1 ml×2

Reaction Components

Component	Volume	Final Concentration
Template DNA	Variable	as required
Forward Primer (10 µM)	1 µl	0.2 µM
Reverse Primer (10 µM)	1 µl	0.2 µM
10×*TransStart® TopTaq* Buffer	5 µl	1×
2.5 mM dNTPs	4 µl	0.2 mM
TransStart® TopTaq DNA Polymerase	0.5-1 µl	1.25-2.5 units
Nuclease-free Water	Variable	-
Total volume	50 µl	-

Thermal cycling conditions

94°C	2-5 min	
94°C	30 sec	30-35 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

Note

- A final concentration of 2 mM $MgSO_4$ is sufficient for most targets amplification. For some targets, more Mg^{2+} may be required.
- For optinal results, we recommend to use the 100 mM $MgSO_4$ stock to prepare a titration from 2 mM to 4 mM (final concentration) in 0.25 mM increments.
- 0.5 µl (2.5 units) enzyme is enough for per 50 µl reaction. For better amplification, up to 1 µl (5 units) enzyme can be used.

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