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CERTIFICATE OF ANALYSIS

TrueStart™ Hot Start *Taq* DNA Polymerase

#EP0612 500 u

Lot: __ **Expiry Date: __**

Concentration: 5 u/µl

Supplied with: 2x1.25 ml of 10X TrueStart™ *Taq* Buffer
2x1.25 ml of 25 mM $MgCl_2$

Store at -20°C

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ISO 9001 ISO 14001
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Description

TrueStart™ Hot Start *Taq* DNA Polymerase is a *Taq* DNA polymerase which has been chemically modified by the addition of heat-labile blocking groups to amino acid residues. The enzyme is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer dimers. The TrueStart™ Hot Start *Taq* DNA Polymerase is instantaneously activated during the initial denaturation step of PCR. The activated enzyme is a functional equivalent of *Taq* DNA Polymerase: it catalyzes 5'→3' synthesis of DNA, has no detectable 3'→5' proofreading exonuclease activity, but possesses low 5'→3' exonuclease activity.

TrueStart™ Hot Start *Taq* DNA Polymerase is designed for Hot Start PCR, a technique that enhances the specificity, sensitivity and yield of DNA amplification (1-5). In addition, the enzyme provides the convenience of room temperature reaction set-up.

Applications

- High throughput hot start PCR.
- RT-PCR.
- Highly specific amplification of genomic DNA and cDNA targets up to 3 kb.
- Amplification of low copy DNA targets.
- Real-time PCR.
- Multiplex PCR.
- Generation of PCR products for TA cloning.

Source

E. coli cells with a cloned *pol* gene from *Thermus aquaticus.*

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 74°C.

Enzyme activity is assayed in the following mixture: 25 mM TAPS (pH 9.3 at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dATP, dGTP, dTTP, 0.1 mM dCTP, 0.75 mM activated salmon milt DNA and 0.4 MBq/ml [^{3}H]-dCTP. The enzyme is activated by heating for 3 hours at 80°C, before the activity is measured.

Storage Buffer

Enzyme is supplied in: 20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Tween 20, 0.5% (v/v) Nonidet P40 and 50% (v/v) glycerol.

10X TrueStart™ *Taq* Buffer

200 mM Tris-HCl (pH 8.3 at 25°C), 200 mM KCl, 50 mM $(NH_4)_2SO_4$.

Inhibition and Inactivation

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02 and 0.01%, respectively (6).
- Inactivated by phenol/chloroform extraction.

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 u of TrueStart™ Hot Start *Taq* DNA Polymerase with 1 µg of pUC19 DNA in 50 µl of TrueStart™ *Taq* buffer containing 2.0 mM $MgCl_2$ for 4 hours at 37°C and 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 10 u of TrueStart™ Hot Start *Taq* DNA Polymerase with 1 µg of digested DNA in 50 µl of TrueStart™ *Taq* buffer containing 2.0 mM $MgCl_2$ for 4 hours at 37°C and 70°C.

Ribonuclease Assay

<0.5% of the total radioactivity was released into the trichloroacetic acid-soluble fraction after incubation of 10 u of TrueStart™ Hot Start *Taq* DNA Polymerase with 1 µg of [^{3}H]-RNA in 50 µl of TrueStart™ *Taq* buffer containing 2.0 mM $MgCl_2$ for 4 hours at 37°C.

Functional Assay

TrueStart™ Hot Start *Taq* DNA Polymerase was tested for yield and specificity in amplification of a 950 bp DNA fragment of the single copy gene using human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene

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PROTOCOL

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by adding water, buffer, dNTPs, primers and TrueStart™ Hot Start *Taq* DNA Polymerase. Prepare enough master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and add template DNA. Keep all reaction components on ice. Reaction set up can be performed at room temperature.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Add the following components for each 50 µl reaction at room temperature:

10X TrueStart™ Hot Start *Taq* buffer	5 µl
dNTP Mix, 2 mM each (#R0241)	5 µl (0.2 mM of each)
Forward primer	0.1-1 µM
Reverse primer	0.1-1 µM
25 mM $MgCl_2$	1-4 mM*
Template DNA	1 pg - 1 µg
TrueStart™ Hot Start *Taq* DNA Polymerase	1.25-2 u
Water, nuclease-free (#R0581)	to 50 µl
Total volume	**50 µl**

*Volumes of 25 mM $MgCl_2$ solution to be used:

Final concentration of $MgCl_2$, mM	1	1.5	2	2.5	3	4
Volume of 25 mM $MgCl_2$ for 50 µl reaction, µl	2	3	4	5	6	8

3. Gently vortex and spin down the samples.
4. When using thermal cyclers without a heated lid, overlay the reaction mixture with 25 µl mineral oil.
5. Perform PCR using recommended thermal cycling conditions:

Step	**Temperature, °C**	**Time**	**Number of cycles**
Initial denaturation/ enzyme activation	95	1-2 min	1
Denaturation	95	0.5-1 min	30
Annealing	Tm-5	0.5-1 min	
Extension	72	1 min/kb	
Final extension	72	5-15 min	1

References

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2. Kellog, D.E., et al., TaqStart antibody: "Hot start" PCR facilitated by a neutralizing monoclonal antibody directed against *Taq* DNA polymerase, BioTechniques, 16, 1134-1137, 1994.
3. Horton, R.M., Hoppe, B.L., and Conti-Tronconi, B.M., AmpliGrease: "Hot Start" PCR using petroleum jelly, BioTechniques, 16, 42-43, 1994.
4. Dang, C. and Jayasena S.D., Oligonucleotide inhibitors of *Taq* DNA polymerase facilitate detection of low copy number targets by PCR, J. Mol. Biol., 264, 268-278, 1996.
5. Moretti, T., et al., Enhancement of PCR amplification yield ans specificity using AmpliTaqGold™ DNA polymerase, Biotechniques, 25, 716-722, 1998
6. Weyant, R.S., et al., Effect of ionic and nonionic detergents on the *Taq* polymerase, Biotechniques, 9, 309-308, 1990.

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.