Hot start Taq DNA polymerase

Cat No	Pack size	conc
HT0500	500U	5U/ul
HT2500	2500U	5U/ul

Description: Hot start Taq DNA Polymerase for qPCR is designed for Real-Time PCR and Hot-start PCR. A special inhibition the reaction at room temperature until after the first denaturation step. This prevents primer-dimers and other artefacts. The enzyme is a thermostable DNA polymerase that possesses a 5'→3' polymerase activity and a double-stranded specific 5'→3' exonuclease activity. The enzyme consists of a single polypeptide with a molecular weight of 94kDa.

Applications:
- Hot Start and real time PCR
- Multiplex PCR
- Amplification of complex genomic and cDNA templates

Storage buffer: 50mM Tris-HCl pH7.9, 50mM KCl, 0.1mM EDTA, 1mM DTT, 0.5mM PMSF , 50% lycerol.

10X reaction buffer:
buffer A: high quantity (genomic DNA PCR) containing 15mM $MgCl_2$.
buffer B: high sensitivity (RT-PCR) containing 15mM $MgCl_2$

Unit description: one unit is defined as the amount of enzyme that will incoporate 10n mole of dNTP into acid-insoluble material in 30 minutes at 74oC. The reaction conditions are: 50mM Tris-HCl pH8.8, 50mM NaCl, 5mM MgCl2, 200uM each of dATP, dCTP, dGTP, H3dTTP, 10 ug activated calf thymus DNA and 0.1mg/ml BSA in a final volume of 50 ul.

Storage:50% glycerol (v/v), 20 mM Tris-HCl pH 8.7 at -20ºC, 100 mM KCl, 0.1 mM EDTA.

Source: *E coli clone*

Quality control: The enzyme is free of nicking and priming activities, exonucleases and non-specific endonucleases. SDS/PAGE - 95 kD band. Activity and stability tested via thermo-cycling. The error rate per nucleotide per cycle is ~ 2.5 x 10^{-5}; the accuracy is ~ 4 x 10^4. Estimated half life at 95ºC is 1.5 hours.

PCR reaction mix:

Component	Volume
Hot start Taq	0.5-1ul
10X buffer	10 ul
10mM dNTP	2 ul
Primer1 (20 pmol)	2-4 ul
Primer2 (20 pmol)	2-4 ul
template	1-10 ul
ddH_2O	Up to 100 ul
Total	100 ul

PCR cycles

Step	Temperature	Time	Cycle
Initial denaturation	94-95ºC	10 mins	1
Denaturation	94-95ºC	10-60sec	25-35
Annealing	50-68ºC	10-30sec	
Extension	72ºC	1min/1kb	
Final extension	72ºC	1-10 mins	1

IMPORTANT: Annealing temperature should be 2-6ºC lower than the primer melting temperature.

Shipping and Storage conditions:
Shipping and temporary storage at -20 and for up to 1 month at room temperature has no detrimental effects on the quality of ZymTaq DNA polymerase.

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