BioTherm™ Taq DNA Polymerase

CAT. NO. GC-002-0500, 500 units

APPLICATION

One of the best performance standard Taq polymerases for robust DNA amplification. It can amplify genomic fragment up to 5 kb and plasmid DNA up to 10 kb.

DESCRIPTION

BioTherm™ Taq DNA polymerase is a thermostable DNA polymerase purified from the Thermus aquaticus strain in accordance with the procedures developed by Kaledin for the isolation of thermostable enzymes processing DNA polymerase activity from thermophilic bacteria. Amplification of DNA fragments (100 bp to 10kb) can be achieved with this enzyme. The enzyme has both 5'-3' polymerase and 5'-3' exonuclease activities. BioTherm™ Taq can add a single template-directed deoxyadenosin (A) residue to the 3' end of duplex PCR products.

CONCENTRATION

5 units/µl

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acidinsoluble form in 30 minutes at 72ºC under the assay conditions (25 mM TAPS (tris-(hydroxy-methyl)-methyl-amino-pro panesulfonic acid, sodium salt) pH 9.3 (at 25ºC); 50 mM KCl; 2 mM $MgCl_2$; 1 mM ß-mercaptoethanol) and activated calf thymus DNA as substrate.

STORAGE BUFFER

10 mM K-phosphate buffer pH 7.0, 100 mM NaCl, 0.5 mM EDTA; 1 mM DTT, 0.01% Tween 20; 50% glycerol (v/v).

STORAGE TEMPERATURE

-20°C.

10X REACTION BUFER

10 x PCR buffer (BioTherm): 160 mM (NH 4) 2 SO 4 , 670 mM Tris-HCl pH 8.8 (at 25ºC), 15 mM MgCl 2 , 0.1% Tween 20.

QUALITY CONTROL

Activity, non-specific endonucleases, nickases, and exonucleases.

Standard PCR Protocol

A standard PCR procedure is provided for amplification of most target sequences. However, we suggest each given PCR application should be optimized accordingly to achieve the best result, especially for repetitive diagnostic or analytic procedures in which optimal performance is necessary.

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Component	Amount (for 100 µl reaction)	Final Concentration
H_2O (DNase free)	- µl	
10x PCR Buffer	10 µl	1x
dNTP (25 mM of each dNTP)	0.8 µl	0.2 mM of each dNTP
DNA template	- µl	1 pg ~1 µg
Primer 1 (10 µM)	2 µl	0.2 µM
Primer 2 (10 µM)	2 µl	0.2 µM
Taq DNA polymerase (5 U/µl)	0.4 - 1 µl	2 - 5 Units
PCR Cycling Program	94 °C x 4' (94 °C x 30", 55 °C x 30", 72 °C x 1'), 30 cycles 72 °C x 5'	

Optimization of PCR Compositions

The use of PCR to amplify a single or multiple target sequences (amplicons) often produces unwanted products or no product at all. So the optimization of PCR conditions by changing the composition of one or several of the key variables becomes necessary. Among all the variables that contribute to primer-template fidelity and primer extension, the Mg^{2+} concentration and the annealing temperature are the most important.

1. **Magnesium Concentration:** the Mg^{2+} concentration varies in different PCR buffer systems. For amplification of genomic DNA sequences, the optimal Mg^{2+} concentration could be determined by adding Mg^{2+} to a series of final concentrations of 1.5 mM to 4.5 mM. Normally lower Mg^{2+} concentration increases the specificity of the amplification products but with lower yield; higher Mg^{2+} concentration increases the yield of non-specific amplification products but promotes misincorporation.
2. **Template DNA:** the amount of DNA template required varies according to the type of the DNA to be amplified. Generally, 100 ng to 1000 ng of genomic DNA is recommended for a total PCR reaction volume of 100 µl. Less DNA (0.01-50 ng) can be used for amplification of plasmid DNA, purified DNA, and viral DNA.
3. **dNTP Concentration:** the concentration of each dNTP in the reaction mixture is usually 200 µM. The four dNTPs should be used at the equivalent concentration to minimize the misincorporation errors. Both the specificity and the fidelity of PCR are increased by using lower dNTP concentration (10-50 µM) than the recommended 200 µM.
4. **Primer Concentration:** the primer concentrations between 0.1 µM and 1 µM are generally optimal. Higher primer concentration may promote msipriming and accumulation of nonspecific amplification products.
5. **Taq DNA Polymerase Concentration:** the amount of Taq DNA polymerase varies depending on the length of template to be amplified. Successful amplification can usually be achieved using 2-5 units of enzyme/100 µl reaction volume (higher for long template amplification).
6. **Primer Annealing:** the typical annealing temperature is between 55 °C and 72 °C. The optimal annealing temperature could be calculated by "Tm - 5 °C".
7. **Primer Extension:** usually the extension step is performed at 70~75°C. The rate of DNA synthesis by Taq DNA Polymerase is the highest at this temperature (2~4 kb/min), and a 1 minute extension time is sufficient for the synthesis of PCR fragments up to 2 kb. When larger DNA fragments are amplified, the extension time is usually increased by 1 minute for each additional 1kb.

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