

BioTherm DNA Polymerase

CAT. NO. DP-002-0020, 200 units

DP-002-0050, 500 units

DP-002-0500, 5000 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 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 can add a single template-directed deoxyadenosin (A) residue to the 3' end of duplex PCR products.

CONCENTRATION

5 units/ μ l

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acidinsoluble form in 30 minutes at 72°C under the assay conditions (25 mM

TAPS (tris-(hydroxy-methyl)-methyl-amino-propanesulfonic acid, sodium salt) pH 9.3 (at 25°C); 50 mM KCl; 2 mM MgCl₂; 1 mM β -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 BUFFER

10 x PCR buffer (BioTherm): 160 mM (NH₄)₂SO₄, 670 mM Tris-HCl pH 8.8 (at 25°C), 15 mM MgCl₂, 0.1% Tween 20.

QUALITY CONTROL

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

Set-up of a Standard PCR Reaction	
Component	Amount
ddH ₂ O	- μl
10x buffer	10 μl
dNTP (25 mM)	0.8 μl
DNA template	1 ng-1 μg
Primer 1 (20 μM)	5 μl
Primer 2 (20 μM)	5 μl
BioTherm DNA polymerase (5 units/μl)	0.4-2 μl
Total volume	100 μl

Note:

1. Mg²⁺ concentration varies in different PCR reaction buffer system. For amplification of genomic DNA sequence, the optimal Mg²⁺ should be determined by adding Mg²⁺ to a final concentration of 1.5 mM to 4.5 mM;
2. The amount of DNA template required varies depending on the type of DNA being amplified. Generally, 50 ng to 1000 ng of genomic DNA is recommended; Less DNA (1 ng-100 ng) can be used for amplification of plasmid DNA, purified DNA, and virus DNA;
3. Primer concentration between 0.2 μM and 1 μM are recommended (approximately 100 ng to 250 ng for typical 18- to 25-mer oligonucleotide primers in 100 μl reaction volume).
4. 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).

Standard Temperature cycling Program		
1. 93°C	3 min	
2. 93°C	30-60 sec	25-35 Cycles
55-65°C	30-60 sec	
72°C	2 min (add 1 min per kb target sequence length)	
3. 72°C	5 min	
4. 4°C		

Note:

1. High quality thin-wall PCR tubes, strips, and plates are highly recommended for getting a consistent and duplicable PCR results. This is particularly important when amplification volume is less than 20 μl. EU thin-wall tubes can produce the most reliable results and the amplification volume can be as low as 5 μl.
2. The typical annealing temperature is between 55 °C and 72 °C. The optimal annealing temperature could be calculated by "T_m - 5 °C".