Elite™ High Fidelity DNA Polymerase

Catalog No. DP-003-0250, 250 Units
DP-003-0500, 500 Units

APPLICATION

Due to its 3'-5' proofreading exonuclease activity, Elite™ high fidelity DNA polymerase is strongly suggested for extremely high fidelity PCR applications, such as subcloning of PCR amplified cDNA, cDNA library construction, etc.

DESCRIPTION

Elite™ High Fidelity DNA Polymerase is a thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proofreading exonuclease activities. It is isolated from the hyperthermophilic marine archae Pyrococcus furiosis (Pfu). Base misinsertions that may occur during polymerization are rapidly excised by the proofreading activity of the polymerase. Elite™ DNA Polymerase generates blunt-ended PCR fragments.
Elite™ DNA Polymerase can amplify fragments up to 5 kb at extremely high fidelity. A mixture of BioTherm™ DNA Polymerase with Elite™ DNA Polymerase provides more robust synthesis of longer (>5 kb) amplification products.

CONCENTRATION

5 units/µl.

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C under the assay conditions (25 mM TAPS (tris-(hydroxymethyl)methyl-amino-propane-sulphonic acid, sodium salt) pH 9.3 (at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 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)

5X REACTION BUFFER

83 mM $(NH_4)_2 SO_4$, 350 mM Tris-HCl pH 8.8 (at 25°C), 22 mM $MgCl_2$, 0,75% Triton X100, 100 mM KCl.

STORAGE TEMPERATURE

Store at -20°C in a constant temperature freezer.

REFERENCES

1. Fiedler, et al. (2008). *J Bacteriol* 190: 6559—6567.
2. Jahromi, et al. (2009). *Hybridoma (Larchmt).* 28: 305--313.
3. Moreno, et al. (2008). *J Biol Chem.* 283: 9633--9641.

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GUIDELINE OF PCR AMPLIFICATION:

Set-up of a Standard PCR Amplification	
Component	**Amount**
ddH_2O	**- 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**
Taq DNA polymerase (5 units/µl)	**0.4-2 µl**
Total volume	**100 µl**

Note:

1. Mg^{2+} concentration varies in different PCR reaction buffer system. For amplification of genomic DNA sequence, the optimal Mg^{2+} should be determined by adding Mg^{2+} 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 PCR Temperature cycling Program		
1. 94°C	**4 min**	
2. 94°C	**30-60 sec**	
55-65°C	**30-60 sec**	**25-35 Cycles**
72°C	**1 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 "Tm - 5 °C".