EU-Taq DNA Polymerase

CATALOG NO. DP-005-0050, 500 U

APPLICATION

- High-throughput genotyping and screening
- Multiplexed genotyping
- Quantitative Real-time PCR
- Nested PCR

DESCRIPTION

EU-Taq is a thermostable DNA polymerase isolated from a strain of Thermus sp. It has an increased half-life of 3 hours at 95°C and 10 fold higher fidelity than other competitive Taqs (error rate: 1 x 10^{-6}). EU-Taq has excellent performance in genomic DNA amplification with variety of primer sets.

CONCENTRATION

5 units/μl

QUALITY CONTROL

EU-Taq is highly purified without any detectable endonucleases, exonucleases and "nicking activity". The enzyme is also bacterial DNA free (no amplification of bacterial gene detected after 35 cycles).

UNIT DEFINITION

One unit incorporates 10 nmole of dNTP into acid-insoluble material in 30 min. at 74 °C

10 X REACTION BUFFER (without Mg^{2+})

670 mM Tris-HCl (pH 8.8), 160 mM $(NH_4)_2SO_4$, 0.1% Tween 20.

MAGNESIUM CHLORIDE

100 mM $MgCl_2$. The final magnesium concentration may be variable according to individual applications. In general, 2.5 mM $MgCl_2$ is recommended.

STORAGE BUFFER

20 mM Tris-HCl buffer (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 0.5 mM PMSF, 1 mM DTT, 50% glycerol (v/v).

STORAGE TEMPERATURE

Store at -20°C in a constant temperature freezer

CONTENTS

EU-Taq (5 U/μl),	1 vial (100 μl)
10x PCR buffer (no Mg^{2+}),	1 vial (1.8 ml)
100 mM $MgCl_2$,	1 vial (1 ml)

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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.

Component	Amount (for 100 µl reaction)	Final Concentration
H_2O (DNase free)	- µl	
10x PCR Buffer	10 µl	1x
$MgCl_2$ (100 mM)	- µl	1.5 – 3.5 mM
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.

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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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