



EU-Taq DNA Polymerase

CATALOG NO. DP-005-0050, 500 units, 5 units/ μ l
DP-005-0500, 5000 units, 5 units/ μ l

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: 1×10^{-6}).

EU-Taq has excellent performance in genomic DNA amplification with variety of primer sets.

CONCENTRATION

5 units/ μ l

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 (w/o Mg²⁺)

200 mM Tris-HCl (pH 8.75), 100 mM KCl, 100 mM (NH₄)₂SO₄, 1% Triton X100, 1mg/ml BSA. Buffer is optimized for use with 200 μ M dNTPs (Cat# NT-013-0050).

MAGNESIUM SULFATE

20 mM MgSO₄. The final magnesium concentration may be variable according to individual applications. In general, 2.5 mM MgSO₄ 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.

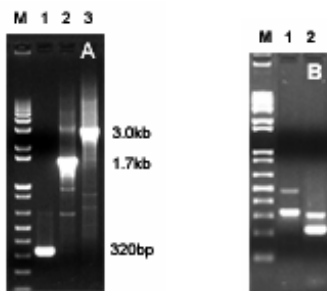


Figure A, Amplification of genomic DNA using *EU-taq* with various primer set. PCR conditions: 94 °C x 2'; 94 °C x 45", 55 °C x 45", 72 °C x 30"-3', for 30 cycles.

Figure B, Duplex PCR amplification. Lane1, allele specific genotyping of human PAX6 mutation, 490bp-mutant band, 320bp-wild type band; Lane2, two primer sets targeted to ALDH3 gene (200bp) and SHH (Sonic Hedgehog) gene. PCR conditions: 94 °C x 2'; 94 °C x 30", 55 °C x 30", 72 °C x 30", for 30 cycles.

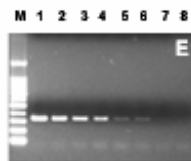


Figure E, Titration of plasmid DNA concentration in 25 µl PCR reaction. Lane M, 100-bp marker, lane1, 1 ng; lane2, 0.1 ng; lane3, 0.01 ng; lane4, 5 pg; lane5, 1 pg; lane6, 0.5 pg; lane7, 0.1 pg; lane8, 0.01 pg. PCR conditions: 94 °C x 2'; 94 °C x 30", 55 °C x 30", 72 °C x 30", for 25 cycles.

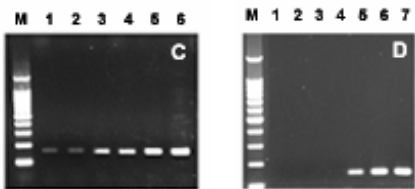


Figure C and D, Titration of enzyme concentration in 25 µl PCR reaction (C, *EU-taq*, D, *Taq* from other company). Lane M, 100-bp maker, lane1, 0.01 U; lane2, 0.02 U; lane3, 0.1 U; lane4, 0.2 U; lane5, 0.5 U; lane7, 1 U. PCR conditions: 94 °C x 2'; 94 °C x 30", 55 °C x 30", 68 °C x 30", for 25 cycles.

GUIDELINE OF PCR AMPLIFICATION

Set-up of a Standard PCR Amplification	
Component	Amount
ddH ₂ O	- μl
10x buffer	10 μl
MgSO ₄ (20mM)	10-15μl (2 mM-3mM)
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. 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 3.5 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 55-65°C 72°C	30-60 sec 30-60 sec 1 min (add 1 min per kb target sequence length)	25-35 Cycles
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".