Taq DNA Polymerase

Ref : NB-03-0083 500 U
NB-03-0084 500 U
NB-03-0085 1 000 U
NB-03-0086 1 000 U
NB-03-0087 18 000 U

Description

Taq DNA Polymerase is a thermostable recombinant DNA polymerase derived from thermophilic bacterium *Thermus aquaticus*. Its molecular weight is 94 kDa. Taq DNA Polymerase can amplify DNA target up to 5 kb （simple template). The elongation velocity is 0.9~1.2kb/min (70~75°C). It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product.

Applications

• PCR amplification of DNA fragments as long as 5 kb
• DNA labeling
• DNA sequencing
• PCR for cloning

Contents

NB-03-0083	
Taq DNA Polymerase (5 U/µl)	100 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml
6X Loading Buffer	1 ml

NB-03-0084*	
Taq DNA Polymerase (5 U/µl)	100 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml
dNTPs (each 2.5 mM)	1 ml
6X Loading Buffer	1 ml

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NB-03-0085	
Taq DNA Polymerase (5 U/µl)	200 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml ×2
6X Loading Buffer	1 ml

NB-03-0086*	
Taq DNA Polymerase (5 U/µl)	200 µl
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml ×2
dNTPs (each 2.5 mM)	1 ml ×2
6X Loading Buffer	1 ml

* with dNTPs

NB-03-0087	
Taq DNA Polymerase (5 U/µl)	100 µl ×36
10X PCR Buffer (Mg^{2+} Plus)	1.4 ml ×36

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

20mM TrisCl (pH8.0), 100mM KCl, 3mM MgCl2 1mM DTT, 0.1% NP-40 ,0.1% Tween20, 0.2mg/ml BSA, 50% (v/v) glycerol

10X Taq Buffer with Mg^{2+}

120mM Tris-HCl (pH 8.8), 500mM KCl, 1%Triton-X-100, 100mM Lycine, 25mM MgSO4

Note

- Recombinant Taq DNA Polymerase is the enzyme of choice for most PCR applications.
- The half-life of enzyme is >40 minutes at 95°C.
- The error rate of Taq DNA Polymerase in PCR is $2.2x10^{-5}$ errors per nt per cycle; the

accuracy (an inverse of the error rate) an average number of correct nucleotides incorporated before making an error, is $4.5x10^4$.

- Taq DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1 µg-1 µg
Plasmid DNA	0.5 ng-5 ng
Phage DNA	0.1 ng-10 ng
E.coli genomic DNA	10 ng-100 ng

Basic PCR Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Taq DNA Polymerase, primers, Mg2+, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

Components	Volume for 50µl of reaction mixture	Final Concentration
10X Taq Buffer (Mg^{2+} Plus)	5 µl	1 ×
dNTPs (each 2.5 mM)	4 µl	0.2 mM each
Primer mix	variable	0.4 – 1 µM each
Template DNA	variable	10 pg – 1 µg
Taq DNA Polymerase (5 U/µl)	0.25–0.5 µl	1.25-2.5 U
Sterile deionized water	to 50 µl	

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94℃	3 min
25-35 cycles	94℃ 55-68°C 72°C	30 s 30 s 1 min
Final extension	72℃	10 min

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Notes on cycling conditions :

- Initial denaturation can be performed over an interval of 1-5 min at 95℃ depending on the GC content of template.
- Optimal annealing temperature is 5℃ lower than the melting temperature of primer-temperature DNA duplex. If nonspecific PCR products are obtained optimization of annealing temperature can be performed by increasing temperature stepwise by 1-2℃.
- The time of the final extension step can be extended for amplicons that will be cloned into T/A vectors.

Store all components at –20°C