Taq DNA polymerase Economy (+dNTPs), with Enhancer for High GC template and Robust buffer

02-003, 200 U (5U/μl)

***Thermus aquaticus* DNA polymerase (*Taq* DNA polymerase)** was expressed in *E. coli* in large quantities and highly purified. The enzyme has thermostable DNA polymerase activity and the MW is 94 kDa. **This enzyme kit is especially suitable for PCR reactions with high GC template due to Ehancer for high GC templates and Robust buffer.**

Applications:

1) High-throughput PCR
2) Colony PCR
3) Incorporation of dUTP, dITP, and fluorescence-labeled nucleotides
4) Primer extension
5) Addition of a single nucleotide (adenosine) at the 3'-blunt ends (for cloning into TA vector)

General composition of PCR reaction mixture (total 50ul)	
Taq DNA polymerase (5 units/ul)	0.25 ul*
10 x Robust Buffer (*Taq*)	5 ul
5 x GC Enhancer solution	10 ul
2.5mM (each) dNTPs	4ul
Template	<500ng
Primer 1	0.2～1.0uM (final conc.)
Primer 2	0.2～1.0uM (final conc.)
Sterile distilled water	up to 50ul

*Use of excess amount of the enzyme is not recommended.

Storage Conditions

Taq DNA polymerase in 20mM Tris-HCl (pH 8.0), 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% glycerol, 0.5% Tween20, 0.5% Igepal CA-630.,**Store at -20℃**

Concentration: 5 units/ul, where one unit is defined as the amount of enzyme that can incorporate 10 nmols of total dNTPs into an acid-insoluble material in 30 minutes at 74℃ when activated salmon sperm DNA was used as template/primer.

Quality Assurance: Greater than 95% purity as determined by SDS-PAGE (CBB staining) (Fig.1)
The absence of endonucleases and exonucleases was confirmed.

PCR Test: Good amplification result was obtained in PCR reaction using λDNA as a template up to 14 kB (Fig.2).

Reagents Supplied with Enzyme:

1. 10 x Robust Buffer (*Taq*)
2. 5 x GC Enhancer
3. 2.5mM(each) dNTPs

Cautions for usage of Robust Buffer (*Taq*) without GC Enhancer

Robust Buffer induces maximum enzymatic activity. Therefore, cares should be taken to avoid production of undesirable smear bands in gel electrophoresis analysis by longer than optimal reaction time. We recommend about 5 to 10 seconds / kb elongation time for template up to 8 kb, and about 15 seconds / kb for up to 14 kb. We will recommend roughly the same elongation time to be set with 2-step PCR (shuttle PCR) and 3-step PCR. Extend the elongation time by short steps when amplification is not seen.

The results of your experiments can be observed more rapidly by adopting 2-step PCR.

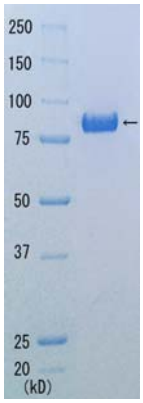


Fig.1 SDS-PAGE analysis of *Taq* DNA polymerase

Protocols for PCR:

Examples of PCR coditions **without GC Enhancer** for the amplification of various sizes of λ DNA **(Results shown in Fig.2)**

2kb, 4kb

94 ° C 1min
95 ° C 5sec
65 ° C 20sec] 25cycles

6kb

94 ° C 1min
95 ° C 5sec
65 ° C 1min] 25 cycles

8kb

94 ° C 1min
95 ° C 5sec
65 ° C 1min 20sec] 25 cycles

10kb, 12kb

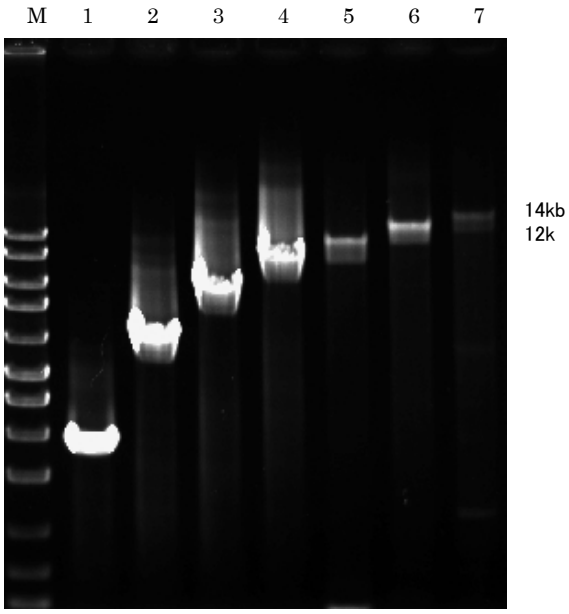
94 ° C 1min
98 ° C 5sec
68 ° C 3min] 30 cycles
72 ° C 3min

14kb

94 ° C 1min
98 ° C 5sec
68 ° C 4min] 30 cycles
72 ° C 4min

Fig. 2 PCR products obtained by using Robust buffer (agarose gel electrophoresis)

M	markers
1	2kb
2	4kb
3	6kb
4	8kb
5	10kb
6	12kb
7	14kb

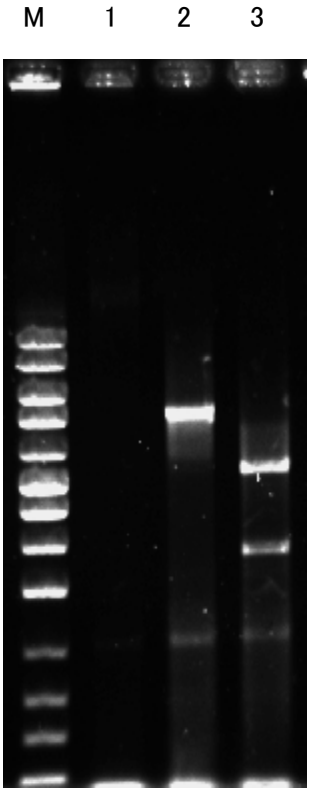


Examples of PCR coditions with GC Enhancer for the amplification of the adenylate cyclaseA gene from *Bordetella pertussis* (ToHAMA I) genomic DNA (GCcontent 67%) **(Results shown in Fig.3)**

98 ° C 2min
98 ° C 5sec
68 ° C 1min] 14 cycles
98 ° C 5sec
68 ° C * 1min] 16 cycles
72 ° C 3min

* decrease 0.5 ° C / cycle

Fig.3 Effect of the Enhancer on the efficiecy of POR with high GC template (the adenylate cyclase gene from *Bordetella pertussis*; 67% GC, 6 kb)



M Marker
1 without GC Enhancer
2 with GC Enhancer
3 NcoI digestion of the PCR product

The adenylate cyclase A gene has a unique NcoI site. The sizes of the digested fragments corresponded to those expected from the physical map.

GC Enhancer consists of the mixture of reagents that decrease a melting point of DNA and stabilize DNA -enzyme interaction.

Five-time dilution of 5x Enhancer is the maximum concentration that can be used. Users are recommended to use 10-time dilution and increase the concentrations to 5-time dilution if it is necessary to optimize the PCR reaction.