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

02-003　　200 U　　(5 U/μl)

Storage: Ship at 4℃ or -20℃ and store at -20˚C.

Concentration: 5 units/μl

*Note: 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 ˚C when activated salmon sperm DNA is used as template/primer.

Storage Buffer:

20mM Tris-HCl (pH 8.0), 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% glycerol, 0.5% Tween 20, 0.5% Igepal CA-630.

Supplied Reagents:

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

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)

Background: *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.

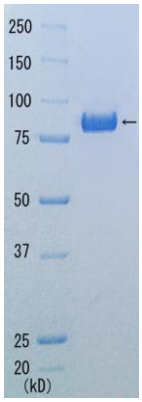
Quality Assurance: Greater than 95% purity as determined by SDS-PAGE (CBB staining) (Fig.1)
The absence of endonucleases and exonucleases was confirmed.

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

Cautions for using Robust Buffer (*Taq*) without GC Enhancer: Robust Buffer induces maximum enzymatic activity. To avoid production of undesirable smear bands in gel electrophoresis analysis, the optimal reaction time is recommended as follows: 1) about 5 to 10 seconds / kb elongation time for template up to 8 kb, and about 15 seconds / kb for up to 14 kb; 2) roughly the same elongation time is set with2-step PCR (shuttle PCR) and 3-step PCR; 3) extend the elongation time by short steps when amplification is not seen. Amplification can be detected more rapidly by adopting 2-step PCR.

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

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



*Taq*DNA polymerase

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

Protocols for PCR

Examples of PCR conditions without GC Enhancer for the amplification of various sizes of λDNA (results shown in Fig.2)

2 kb, 4 kb

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

6 kb

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

8 kb

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

10 kb, 12 kb

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

14 kb

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

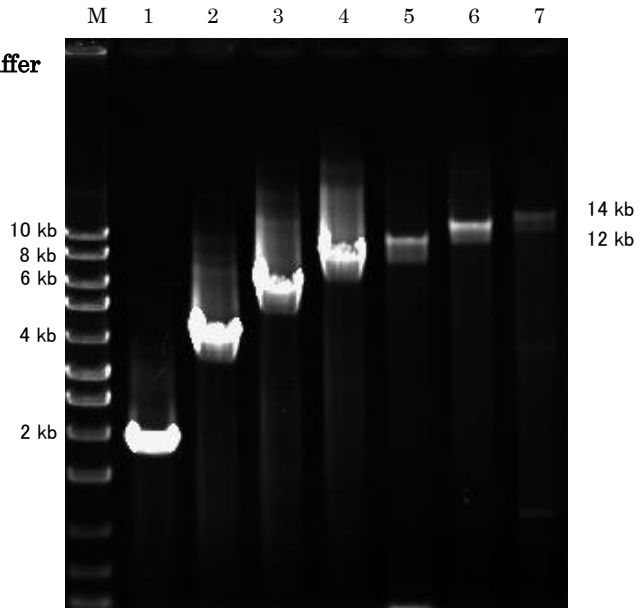


Fig. 2 PCR products obtained by using Robust Buffer (agarose gel electrophoresis)

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

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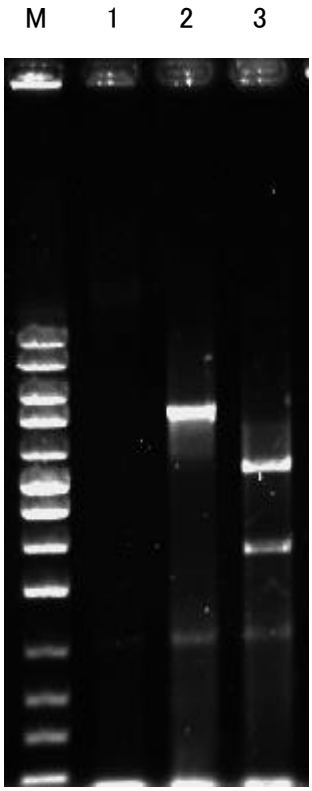
Examples of PCR coditions with GC Enhancer for the amplification of the adenylate cyclaseA gene from *Bordetella pertussis* (ToHAMA I) genomic DNA (GCcontent 67%)(results shown in Fig.3)

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 5 x 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.