Taq DNA polymerase Economy (−dNTPs), with Robust Buffer

02-012, 200 U (5 U/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 is suitable for PCR reactions; capable of amplifying DNA with various primers.

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
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℃

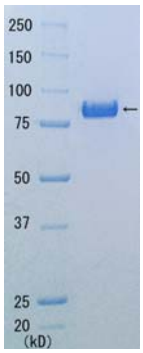
Concentration: 5 units/ul, where one unit is defined as the amount of enzyme that can incorporate 10 nmols of total dNTPs into an acid-insoluble material in 30 minutes at 74℃ when activated salmon sperm DNA was used as template/primer.

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*)



*Taq*DNA polymerase

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

Cautions for usage of Robust Buffer (*Taq*)

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.

Protocols for PCR:

Examples of PCR coditions 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

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

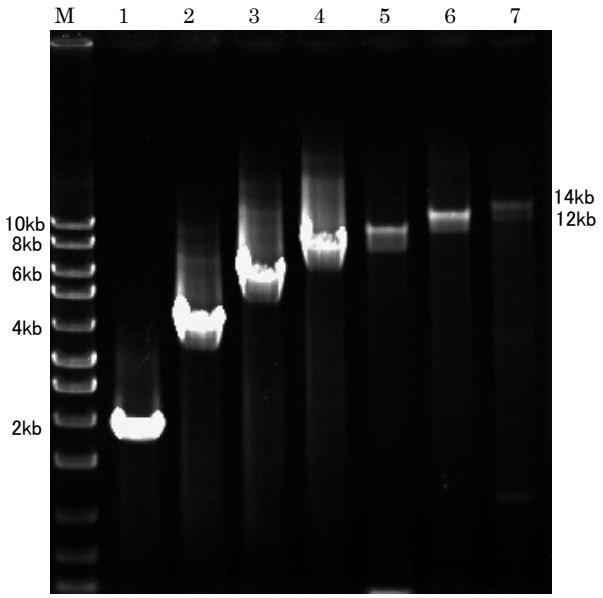


Fig. 2 PCR products obtained by using Robust buffer (agarose gel electrophoresis)