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

02-012 200 U, 02-012-5 5 x 200 U

Storage: 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 Reagent: 10 x Robust Buffer (*Taq*)

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 is suitable for PCR reactions; capable of amplifying DNA with various primers.

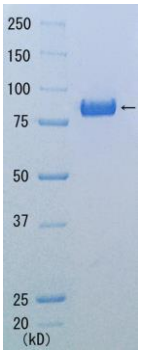
Quality Assurance: Greater than 95% purity as determined by SDS-PAGE (CBB staining) (Fig.1)
The absence of endonucleases and exonucleases was confirmed.

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*): 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
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 for the amplification of various sizes of λDNA (results shown in Fig.2)

2 kb, 4 kb

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

6 kb

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

8 kb

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

10 kb, 12 kb

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

14 kb

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

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

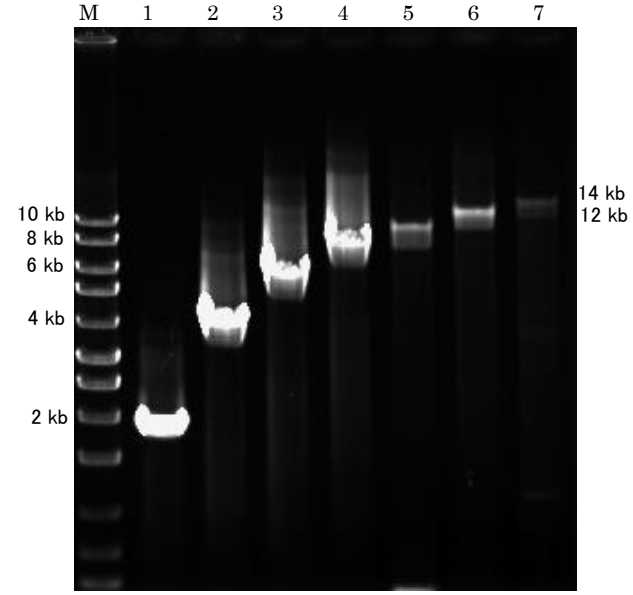


Fig. 2 PCR products obtained by using Robust Buffer (agarose gel electrophoresis)

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