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

02-002　200 U,　02-002-5　5 x 200 U

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℃ 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) 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 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	<500ng
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.

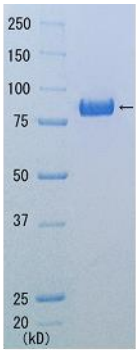


Fig.1 SDS-PAGE analysis of *Taq* DNA polymerase

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	} 25 cycles
65 ˚C	20 sec	

6 kb

94 ˚C	1 min	
95 ˚C	5 sec	} 25 cycles
65 ˚C	1 min	

8 kb

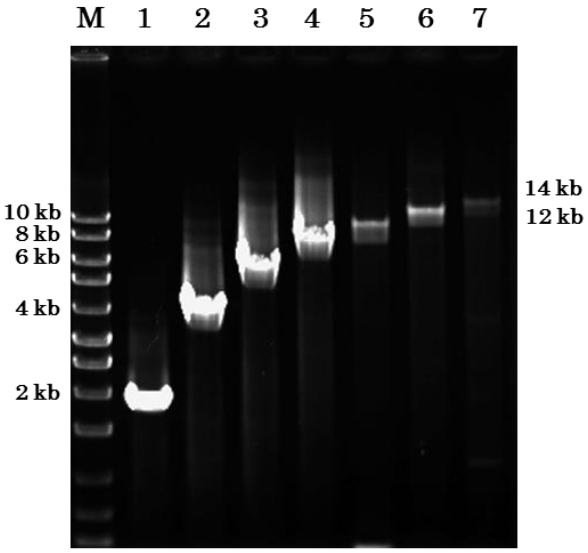
94 ˚C	1 min	
95 ˚C	5 sec	
65 ˚C	1 min 20 sec	} 25 cycles

10 kb, 12 kb

94 ˚C	1 min	
98 ˚C	5 sec	} 30 cycles
68 ˚C	3 min	
72 ˚C	3 min	

14 kb

94 ˚C	1 min	
98 ˚C	5 sec	} 30 cycles
68 ˚C	4 min	
72 ˚C	4 min	



M: marker,
lane 1: 2 kb, lane 2: 4 kb, lane 3: 6 kb,
lane 4: 8 kb, lane 5: 10 kb, lane 6: 12 kb,
lane 7: 14 kb.

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