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

Product code	02-003　200 U　　02-003-5　200 U x 5
Size	200 U
Storage	-20℃
Concentration	5 U/μl
Product Description	*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 Enhancer for high GC templates and Robust buffer.**
Definition of activity	One unit is defined as the amount of enzyme that can incorporate 10nmols of total dNTPs into an acid-insoluble material in 30 minutes at 74℃ when activated salmon sperm DNA was used as template / primer.
Purity	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)
Components	**Taq DNA polymerse (5U/μl)**: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween20, 0.5% Igepal CA-630 (02-Taq 40μl) **10 x Robust Buffer (Taq)** (02-Trb 1.0ml) **5 x GC Enhncer** (02-Enh 2.0ml) **2.5 mM (each) dNTPs** (02-Dnt 640μl)
Application	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 50μl) *Taq* DNA polymerase (5 U/μl) (02-Taq) ※0.25 μl 10x Rebust Buffer (Taq) (02-Trb) 5 μl 5x GC Enhncer (02-Enh) 10 μ1 2.5mM (each) dNTPs (02-Dnt) 4 μl Template <500 ng Primer 1 0.2~1.0 mM (final conc.) Primer 2 0.2~1.0 mM (final conc.) Sterile distilled water up to 50 μl ※ Use of excess amount is not recommended. **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.
Related product	02-013 Taq DNA polymerase with Enhancer for High GC template and Robust Buffer (-dNTPs)

Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.

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Data Images: 02-003 Taq DNA polymerase with Enhancer for High GC template and Robust Buffer (+dNTPs)

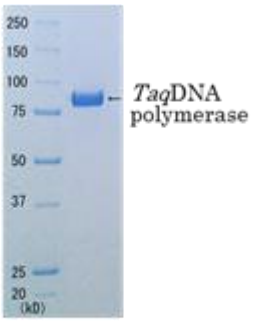


Fig.1 SDS-PAGE of *Taq*DNA polymerase

Protocols for PCR

FIg.2 Examples of PCR conditions without GC Enhancer for the amplification of various sizes of λDNA

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

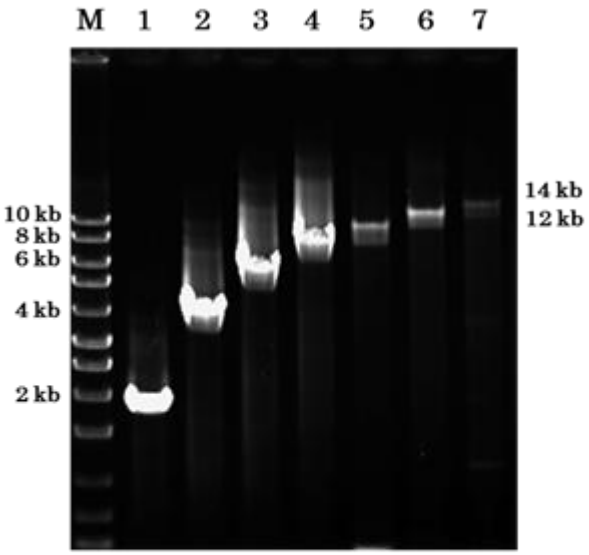
94 ˚C	1 min	
95 ˚C	5 sec	} 25 cycles
65 ˚C	1 min 20 sec	

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)

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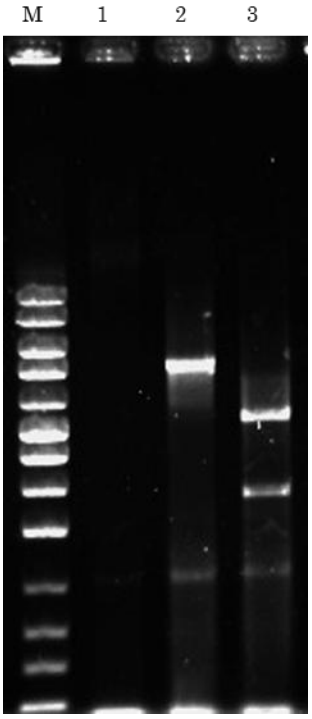
Fig.3 Examples of PCR coditions with GC Enhancer for the amplification of the adenylate cyclaseA gene from *Bordetella pertussis* (ToHAMA I) genomic DNA (GCcontent 67%)

98°C 2min
98°C 5sec
68°C 1min } 14 cycles
98°C 5sec
68°C *1min } 16 cycles *decrease 0.5°C/ cycle
72°C 3min



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.

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)

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