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Inspiration for Life Science

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Catalog No. BAM-02-013-EX

Taq DNA polymerase Economy (-dNTPs), with Enhancer for High GC template and Robust buffer

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.

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 50 ul)	
Taq DNA polymerase (5 units/ul)	0.25 ul*
10 x Robust Buffer (*Taq*)	5 ul
2.5mM (each) dNTPs	4 ul
Template	< 500 ng
Primer 1	0.2～1.0 M (final conc.)
Primer 2	0.2～1.0 M (final conc.)
Sterile distilled water	up to 50 ul

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

Size: 200 UNIT

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.

Form: 20mM Tris-HCl (pH 8.0), 100mM KCl, 0.1mM EDTA, 1mM DTT, 50% glycerol, 0.5% Tween20, 0.5% Igepal CA-630.

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).

Storage: -20°C

Reagents Supplied with Enzyme:

1. 10 x Robust Buffer (Taq)
2. 5 x GC Enhancer

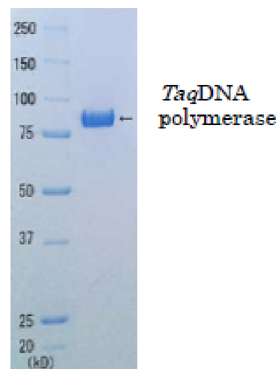


Fig.1 SDS-PAGE analysis of Taq DNA polymerase

Cautions for usage of Robust Buffer (Taq)without GC Enhancer

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.

1. Examples of PCR coditions without GC Enhamcer for the amplification of various sizes of λDNA

Protcol for PCR

2kb, 4kb	6kb	8kb
94°C 1min 95°C 5sec ⎤ 65°C 20sec ⎦ 25cycles	94°C 1min 95°C 5sec ⎤ 65°C 1min ⎦ 25 cycles	94°C 1min 95°C 5sec ⎤ 65°C 1min 20sec ⎦ 25 cycles
10kb, 12kb	**14kb**	
94°C 1min 98°C 5sec ⎤ 68°C 3min ⎦ 30 cycles 72°C 3min	94°C 1min 98°C 5sec ⎤ 68°C 4min ⎦ 30 cycles 72°C 4min	

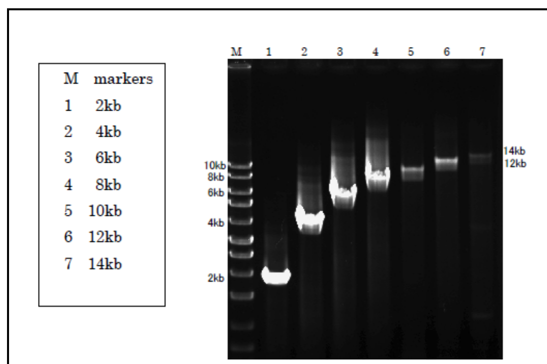
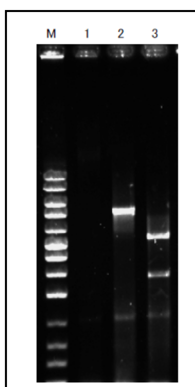


Fig.2 PCR products obtained by using Robust buffer (agarose gel electrophoresis

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

Protcol for PCR

98°C 2min
98°C 5sec ⎤
68°C 1min ⎦ 14 cycles
98°C 5sec ⎤ * decrease 0.5°C / cycle
68°C * 1min ⎦ 16 cycles
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 5x 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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Manufactured by BioAcademia,Inc.

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