PRODUCT INSERT

PUREGENE GENETIX BRAND

Store at -20°C

Taq DNA Polymerase

Cat. No.	Pack Size
PGM040-A	500 U

***Taq* DNA Polymerase** (recombinant)

Component	PGM040-A
Taq DNA Polymerase, 5 U/µL	500 U
10X *Taq* Buffer with KCl	2 X 1.25 mL
10X *Taq* Buffer with $(NH_4)_2SO_4$	2 X 1.25 mL
25 mM $MgCl_2$	2 X 1.25 mL

Description

Taq DNA Polymerase is a highly thermostable DNA polymerase of the thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5'→3' synthesis of DNA, has no detectable 3'→5' exonuclease (proofreading) activity and possesses 5'→3' exonuclease activity. In addition, *Taq* DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant *Taq* DNA Polymerase is ideal for standard PCR of amplicons 5 kb or shorter.

Applications

- Routine PCR amplification of DNA fragments up to 5 kb.
- Generation of PCR product for TA cloning.
- DNA labeling.
- DNA sequencing.

Source

E.coli cells with a cloned *pol* gene from *Thermus aquaticus* YT1.

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 70°C. Enzyme activity is assayed in the following mixture: 67 mM Tris-HCl (pH 8.8 at 25°C), 6.7 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 50 mM NaCl, 0.1 mg/mL BSA, 0.75 mM activated salmon milt DNA, 0.2 mM of each dNTP, 0.4 MBq/mL [3H]-dTTP.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Nonidet® P40, 0.5% (v/v) Tween® 20 and 50% (v/v) glycerol.

10X *Taq* Buffer with KCl

100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 0.8% (v/v) Nonidet P40.

10X *Taq* Buffer with $(NH_4)_2SO_4$

750 mM Tris-HCl (pH 8.8 at 25°C), 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween 20.

Inhibition and Inactivation

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02 and 0.01%, respectively .
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and *Taq* DNA Polymerase. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 ul reaction:

10X *Taq* Buffer	5 µL
dNTP Mix, 2 mM each	5 µL (0.2 mM of each)
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
25 mM $MgCl_2$*	1-4 mM
Template DNA	10 pg - 1 µg
***Taq* DNA Polymerase**	1.25 U
Water, nuclease-free	to 50 µL
Total volume	50 µL

*Volumes of 25 mM $MgCl_2$, required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$, mM	1	1.5	2	2.5	3	4
Volume of 25 mM $MgCl_2$ to be added for 50 µL reaction, µL	2	3	4	5	6	8

3. Gently vortex the samples and spin down.
4. If using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 25 ul of mineral oil.
5. Perform PCR using recommended thermal cycling conditions:

Step	Temperature,°C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	
Annealing	Tm-5	30 s	25-40
Extension	72	1 min/kb	
Final Extension	72	5-15 min	1

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GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free).
- Always perform “no template control” (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Follow general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3’-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conservated nucleotides at the 3’-end.
- When introducing restriction enzyme sites into primers, refer to the table “Cleavage efficiency close to the termini of PCR fragments” located on to determine the number of extra bases required for efficient cleavage.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:
Tm= 4 (G + C) + 2 (A + T),
where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides specialized computer programs are recommended to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA in the 50 ul reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 ug for genomic DNA. Higher amount of template increases the risk of generation of non-specific PCR products. Lower amount of template reduces the accuracy of the amplification.
All routine DNA purification methods are suitable for template preparation. Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

$MgCl_2$ concentration

Due to the binding of Mg^2+ to dNTPs, primers and DNA templates, Mg^{2+} concentration needs to be optimized for maximal PCR yield. The recommended concentration range is 1-4 mM. If the Mg^{2+} concentration is too low, the yield of PCR product could be reduced. On the contrary, non-specific PCR products may appear and the PCR fidelity may be reduced if the Mg^{2+} concentration is too high. If the DNA samples contain EDTA or other metal chelators, the Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds one Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. Due to the binding of Mg2+ to dNTPs, the MgCl2 concentration needs to be adjusted accordingly. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) present in the reaction mixture.

To achieve 0.2 mM concentration of each dNTP in the PCR mixture, use the following volumes of dNTP mixes:

Volume of PCR mixture	dNTP Mix, 2 mM each	dNTP Mix, 10 mM each	dNTP Mix, 25 mM each
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

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Primers
The recommended concentration range of the PCR primers is 0.1-1 uM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.
For degenerate primers higher primer concentrations in the range of 0.3-1 uM are often favorable.

CYCLING PARAMETERS

Initial DNA denaturation
It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient. For GC-rich templates this step should be prolonged up to 10 min. If longer initial denaturation step is required, or the DNA is denatured at a higher temperature, *Taq* DNA Polymerase should be added after the initial denaturation step to avoid a decrease in its activity.

Denaturation
A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min. DNA denaturation can also be enhanced by the addition of either 10-15% glycerol or 10% DMSO, 5% formamide or 1-1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly. In addition, 10% DMSO and 5% formamide inhibit DNA polymerases by 50%. Thus, the amount of the enzyme should be increased if these additives are used.

Primer annealing
The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments. When additives which change the melting temperature of the primer-template complex are used (glycerol, DMSO, formamide and betaine), the annealing temperature must also be adjusted.

Extension
The optimal extension temperature for *Taq* DNA Polymerase is 70-75°C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb.

Number of cycles
The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.
If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension
After the last cycle, it is recommended to incubate the PCR mixture at 72°C for additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors, the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3’-dA tailing of PCR product. If the PCR product will be used for cloning the final extension step can be omitted.

Endodeoxyribonuclease Assay
No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 10 U of *Taq* DNA Polymerase with 1 ug of pUC19 DNA for 4 hours at 37°C.

Exodeoxyribonuclease Assay
No degradation of DNA was observed after incubation of 1 _g of lambda DNA/HindIII fragments with 10 U *Taq* DNA Polymerase for 4 hours at 37°C.

Ribonuclease Assay
No contaminating RNase activity was detected after incubation of 10 U of *Taq* DNA Polymerase with 1 ug of [3H]-RNA for 4 hours at 37°C.

Functional Assay
Taq DNA Polymerase was tested for amplification of 950 bp single copy gene from human genomic DNA and for amplification of cDNA.

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