

PRODUCT INFORMATION

Thermo Scientific ThermoPrime Taq DNA Polymerase

#AB-0301/B 10 x 250 U

Concentration: 5 U/μL
Lot ___ Expiry Date ___

Store at -20 °C



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Ordering Information

Component	#AB-0301/A 250 U	#AB-0301/B 10 x 250 U
ThermoPrime Taq DNA Polymerase, 5 U/μL	50 μL	10 x 50 μL
10X Reaction Buffer IV	1.5 mL	10 x 1.5 mL
25 mM MgCl ₂	1.5 mL	10 x 1.5 mL

Description

Thermo Scientific™ ThermoPrime™ Taq DNA Polymerase is an ultrapure recombinant thermostable Taq DNA polymerase obtained by high level expression of the Taq DNA polymerase gene in *E. coli*. The enzyme exhibits enhanced thermal stability at DNA denaturation temperatures and can be shipped at ambient temperature with no loss of activity. It is licensed and optimized for use in the polymerase chain reaction (PCR) process. ThermoPrime has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

Applications

- Routine PCR amplification of DNA fragments up to 5 kb (1).
- Generation of PCR product for TA cloning.
- DNA labeling (2-4).
- DNA sequencing (5).

Source

E. coli cells with a cloned Taq DNA polymerase gene from *Thermus aquaticus* YT1.

Definition of Activity Unit

One unit of the enzyme is defined as the amount that will incorporate 10 nmol of deoxyribonucleotides into a polynucleotide fraction 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 MgCl₂, 1 mM DTT, 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. Incorporation of [³H]-dTTP is measured adsorbed on DE-81.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.0 at 25 °C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween® 20, 0.5% Nonidet® P40, 50% (v/v) glycerol.

10X Reaction Buffer IV

750 mM Tris-HCl (pH 8.8 at 25 °C), 200 mM (NH₄)₂SO₄, 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 (6).
- 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 ThermoPrime 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 25 μL reaction:

	Volume	Final Concentration
10X Reaction Buffer IV	2.5 μL	1X
dNTP Mix, 2 mM each (#R0241)	2.5 μL	0.2 mM of each dNTP
Forward primer, 10 μM	1.25 μL	0.5 μM
Reverse primer, 10 μM	1.25 μL	0.5 μM
25 mM MgCl ₂	1.5 μL*	1.5 mM*
Template DNA	0.5-10 μL	0.5-125 ng
ThermoPrime Taq DNA Polymerase	0.125 μL	0.625 U
Water, nuclease-free (#R0581)	to 25 μL	
Total volume	25 μL	

*Scale up or down the volume and concentration as appropriate. Volumes of 25 mM MgCl₂, required for specific final MgCl₂ 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 25 μL reaction, μL	1	1.5	2	2.5	3	4

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 half volume of mineral oil.
5. Perform PCR using recommended thermal cycling conditions:

Step	Temperature, °C	Time	Number of cycles
Initial Denaturation	94	2 min	1
Denaturation	94	20 s	30-40
Annealing	50-65	30 s	
Extension**	72	1 min	
Final Extension	72	5 min	1

**Increase length of time in proportion to size of amplicon. ThermoPrime Taq DNA Polymerase extends at approximately 1000 bp/min.

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, (#R0581)).
- Always perform "no template control" (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Use the Thermo Scientific REviewer™ primer design software at www.thermoscientific.com/reviewer or 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 conserved nucleotides at the 3'-end.
- Differences in melting temperatures (T_m) between the two primers should not exceed 5 °C.

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Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (T_m) can be calculated using the following equation:

$$T_m = 4(G + C) + 2(A + T),$$

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, e.g. REViewer (www.thermoscientific.com/reviewer), are recommended to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amount of template DNA in the 25 μ L reaction volume is 0.5-125 ng. 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 e.g., Thermo Scientific GeneJET™ Genomic DNA Purification Kit (#K0721) or GeneJET Plasmid Miniprep Kit (#K0502). 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.

MgCl₂ concentration

Due to the binding of Mg²⁺ to dNTPs, primers and DNA templates, Mg²⁺ concentration needs to be optimized for maximal PCR yield. The recommended concentration range is 1-4 mM. If the Mg²⁺ 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 Mg²⁺ concentration is too high. If the DNA samples contain EDTA or other metal chelators, the Mg²⁺ ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds one Mg²⁺).

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 (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 μ L	5 μ L	1 μ L	0.4 μ L
25 μ L	2.5 μ L	0.5 μ L	0.2 μ L
20 μ L	2 μ L	0.4 μ L	0.16 μ L

Primers

The recommended concentration range of the PCR primers is 0.1-1 μ M. 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 μ M 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 94 °C is sufficient.

Denaturation

A DNA denaturation time of 20 seconds per cycle at 94 °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 (T_m) 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 ThermoPrime Taq DNA polymerase is 70-75 °C. The recommended extension step is 1 min/kb at 72 °C.

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, 30-35 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 (for instance, using Thermo Scientific InsTAclone PCR Cloning Kit (#K1213)), 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 using Thermo Scientific CloneJET PCR Cloning Kit (#K1231), the final extension step can be omitted.

References

- Innis, M.A., et al., PCR Protocols and Applications: A Laboratory Manual, Academic, New York, 1989.
- Celeda, D., et al., PCR amplification and simultaneous digoxigenin incorporation of long DNA probes for fluorescence *in situ* hybridization, BioTechniques, 12, 98-102, 1992.
- Finckh, U., et al., Producing single-stranded DNA probes with the Taq DNA polymerase: a high yield protocol, BioTechniques, 10, 35-39, 1991.
- Yu, H. et al., Cyanine dye dUTP analogs for enzymatic labeling of DNA probes, Nucleic Acids Res., 22, 3226-3232, 1994.
- Innis, M.A., et al., DNA sequencing with *Thermus aquaticus* DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA, Proc. Natl. Acad. Sci. USA, 85, 9436-9440, 1988.
- Weyant, R.S., et al., Effect of ionic and nonionic detergents on the Taq polymerase, Biotechniques, 9, 309-308, 1990.

CERTIFICATE OF ANALYSIS

Deoxyribonuclease Assay

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

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded and double-stranded 5'-[³²P]-labeled oligonucleotides was observed after incubation with 10 U of ThermoPrime Taq DNA Polymerase at 37 °C for 4 hours.

Ribonuclease Assay

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

Functional Assay

ThermoPrime Taq DNA Polymerase was tested for amplification of 956 bp single copy gene from human genomic DNA and for amplification of cDNA.

Quality authorized by:  Jurgita Zilinskiene

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