Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Thermo-Start *Taq* DNA Polymerase

#AB-0908/B **10 x 250 U**

Concentration: 5 U/µL

Lot __ **Expiry Date __**

Store at -20°C

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www.thermoscientific.com/onebio

Ordering Information

Component	#AB-0908/B 10 × 250 U
Thermo-Start *Taq* DNA Polymerase	10 × 50 µL
10X Thermo-Start PCR Buffer	10 × 1.5 mL
25 mM $MgCl_2$	10 × 1.5 mL

Rev.8 53

Description

Thermo Scientific Thermo-Start *Taq* DNA Polymerase is a chemically modified version of Thermo Scientific ThermoPrime *Taq* DNA Polymerase. It is completely inactive at room temperature, preventing the formation and subsequent amplification of non-specific products. The enzyme requires an **activation step at 95 °C for 15 minutes.** Thermo-Start has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

Applications

- Hot start PCR.
- RT-PCR.
- Highly specific amplification of complex genomic and cDNA templates up to 3 kb.
- Generation of PCR product for TA cloning.
- Amplification of low copy DNA targets.
- Real-time PCR.
- Multiplex PCR.

Source

E.coli cells with a cloned *Taq* DNA polymerase 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 in 30 min at 74°C.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 9.2 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.

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, $MgCl_2$, primers and Thermo-Start *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. Keep all reaction components on ice. Reaction set up can be performed at room temperature.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Add the following components for each 25 µL reaction:

	Volume	Final concentration
10X Thermo-Start PCR Buffer	2.5 µL	1X
dNTP Mix, 2 mM (#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
Thermo Start *Taq* DNA Polymerase (5 u/µL)	0.125 µL	0.625 U
Water, nuclease-free (#R0581)	to 25 µL	
Total volume	25 µL	

*Scale up or down the volumes and concentration as appropriate.
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 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	95	15 min	1
Denaturation	95	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. Thermo-Start *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 conservated nucleotides at the 3'-end.
- Differences in melting temperatures (Tm) 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 (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, 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 µL reaction volume is 0.5 – 125 ng. Higher amounts of template increases the risk of generation of non-specific PCR products. Lower amounts 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.

$MgCl_2$ 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 Mg^{2+} to dNTPs, the $MgCl_2$ 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 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

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. 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 µM are often favorable.

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

Thermo-Start Taq DNA Polymerase requires an activation step at 95 °C for 15 min. For extra stringency, the enzyme can be activated gradually during the PCR in a series of steps. The initial activation step is replaced by longer (2 min) denaturation steps for the first 7-8 cycles of the reaction.

Denaturation

A DNA denaturation time of 20 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 Thermo-Satrt *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.

CERTIFICATE OF ANALYSIS

Deoxyribonuclease Assay

No degradation of single-stranded and double-stranded [^{33}P]-labeled oligonucleotides was observed after incubation with 10 U of Thermo-Start *Taq* DNA Polymerase for 4 hours at 37 °C in 1X Thermo-Start PCR Buffer.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 160 ng of 2 kb RNA transcript with 25 U of Thermo-Start *Taq* DNA Polymerase for 4 hours at 37°C.

Functional Assay (PCR)

Thermo-Start *Taq* DNA Polymerase was tested functionally for end-point PCR amplification of a 1988 bp DNA fragment from 50 ng of human genomic DNA in a 25 µL PCR mixture.

Functional Assay (qPCR)

Thermo-Start *Taq* DNA Polymerase was tested functionally for quantitative PCR amplification of DNA fragments from 100 pg – 100 ng of human genomic DNA in a 25 µL PCR mixture.

Quality authorized by: Jurgita Zilinskiene