Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific DreamTaq Hot Start DNA Polymerase

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Lot: __ **Expiry Date:** __

Ordering Information

Catalog No.	DreamTaq Hot Start DNA Polymerase, 5 U/µL	10X DreamTaq Buffer*
EP1701	200 U	1.25 mL
EP1702	500 U	2 × 1.25 mL
EP1703	2500 U	10 × 1.25 mL
EP1704	4 × 2500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

Store at –20°C

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For Research Use Only. Not for use in diagnostic procedures.

DESCRIPTION

Thermo Scientific™ DreamTaq™ Hot Start DNA Polymerase is an enhanced hot start *Taq* DNA polymerase optimized for most PCR applications. It ensures higher sensitivity, specificity, and yields compared to conventional hot start *Taq* DNA polymerase. It is capable of amplifying long amplicons such as 6 kb genomic DNA and 20 kb λ DNA.

DreamTaq Hot Start DNA Polymerase combines *Taq* DNA polymerase and a specific antibody that inhibits the DNA polymerase activity at ambient temperatures, thus preventing the amplification of non-specific products. At polymerization temperatures, the antibody molecule is released, rendering the polymerase fully active.

DreamTaq Hot Start DNA Polymerase uses the same reaction set-up and cycling conditions as conventional *Taq* DNA polymerases, but the antibody-based hot start allows the reactions to be set up at room temperature. Because the enzyme is supplied with the optimized DreamTaq buffer, which includes 20 mM $MgCl_2$, extensive optimization of reaction conditions is not required.

DreamTaq Hot Start DNA Polymerase generates PCR products with 3'-dA overhangs. The enzyme tolerates dUTP and can incorporate modified nucleotides.

FEATURES

- High specificity due to antibody based hot start.
- Robust amplification with minimal optimization.
- High yields of PCR products.
- Higher sensitivity compared to conventional hot start *Taq* DNA polymerases.
- Amplification of long targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- Generates 3'-dA overhangs.
- Incorporates dUTP and modified nucleotides.

APPLICATIONS

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

CONCENTRATION

5 U/µL

DEFINITION OF ACTIVITY UNIT

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 74°C.

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10X DREAMTAQ BUFFER

DreamTaq Buffer is a proprietary formulation, which contains KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance of DreamTaq Hot Start DNA Polymerase in PCR. DreamTaq Buffer also includes $MgCl_2$ at a concentration of 20 mM.

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

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. For each 50-µL reaction, add the following components into a thin-walled PCR tube:

10X DreamTaq Buffer*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1–1.0 µM
Reverse primer	0.1–1.0 µM
Template DNA	10 pg–1 µg
DreamTaq Hot Start DNA Polymerase	1.25 U
Water, nuclease-free (#R0581)	to 50 µL
Total volume	50 µL

*10X DreamTaq Buffer contains 20 mM $MgCl_2$, which is optimal for most applications. If further optimization is required, additional $MgCl_2$ can be added to the master mix. The volume of water should be reduced accordingly.

Volumes of 25 mM $MgCl_2$ (#R0971), required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM $MgCl_2$ to be added for 50-µL reaction	0 µL	1 µL	2 µL	4 µL

3. Gently vortex the samples and briefly centrifuge.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Place the reactions in a thermal cycler. Perform PCR using the recommended thermal cycling conditions outlined below:

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

* The recommended extension step is 1 minute for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 minute/kb.

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. Follow the general recommendations below to lower the risk of contamination.

- 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.

DreamTaq Hot Start DNA Polymerase incorporates dUTP; therefore, you can control carry-over contamination using Uracil-DNA Glycosylase (#EN0361).

GUIDELINES FOR PRIMER DESIGN

Use special design software or follow the general recommendations for PCR primer design as outlined below to design optimal primers:

- Use PCR primers that are 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.

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- Avoid self-complementary primer regions, and complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementarity between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- 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 approximate 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, we recommend using specialized computer programs to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amount of template DNA for a 50-µL reaction volume is 1 pg–1 ng for both plasmid and phage DNA, and 100 pg–1 µg for genomic DNA. Higher amounts of template increase the risk of non-specific PCR products. Lower amounts of template reduce 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

DreamTaq Hot Start DNA Polymerase is provided with an optimized 10X DreamTaq Buffer, which includes $MgCl_2$ at a concentration of 20 mM. A final $MgCl_2$ concentration of 2 mM is generally ideal for PCR. $MgCl_2$ concentration can be further increased up to 4 mM by the addition of 25 mM $MgCl_2$ (#R0971).

If the DNA samples contain EDTA or other metal chelators, Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg^{2+}).

dNTPs

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

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, refer to the table below.

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

Use 200 µM of each dNTP. dUTP or dITP can be added up to 200 µM. For longer amplicons, a lower dUTP concentration (20–100 µM) may be required for high yields.

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 and primers used for long PCR, we recommend higher primer concentrations in the range of 0.3–1 µM.

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

DreamTaq Hot Start DNA polymerase is inactive at room temperature during the reaction set up and is activated during the 1–3 minute initial denaturation/enzyme activation step.

It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 60% or less, an initial 1–3 minute denaturation at 95°C is sufficient. For GC-rich templates this step can be prolonged.

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 minutes. DNA denaturation can also be enhanced by the addition of 5–10% glycerol, 5% DMSO, 1% 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.

Note that higher than 10% DMSO or 5% formamide in the reaction mix inhibit DNA polymerases. Therefore, it may be necessary to increase the amount of the enzyme in the reaction if these additives are used.

Primer annealing

The annealing temperature should be equal to 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 that 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 DreamTaq Hot Start DNA Polymerase is 70–75°C. The recommended extension step is 1 minute at 72°C for PCR products up to 2 kb. For longer products, the extension time should be increased by 1 minute/kb. For amplification of templates >6 kb, we recommend reducing the extension temperature to 68°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 is present in the reaction, about 40 cycles are required. For higher template amounts, 25–35 cycles are sufficient.

Final extension

After the last cycle, we recommend incubating the PCR mixture at 72°C for an additional 5–15 minutes to fill in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors such as the Thermo Scientific™ InsTAclone™ PCR Cloning Kit (#K1213), the final extension step may be prolonged to 15 minutes to ensure the complete 3'-dA tailing of the 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.

TROUBLESHOOTING

For troubleshooting, visit **www.thermofisher.com.**

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable conversion of supercoiled plasmid DNA to a nicked form was observed.

Residual Activity Assay

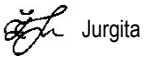
No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs.

E. coli DNA Assay

No detectable *E.coli* DNA was observed.

Functional Assay

Performance in PCR is tested by the amplification of a 594 bp and 7.5 kb fragments of human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

For Research Use Only. Not for use in diagnostic procedures.

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