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PRODUCT INFORMATION

Thermo Scientific DreamTaq DNA Polymerase

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Lot: __ Expiry Date: __

Store at -20°C

Ordering Information

Component	DreamTaq DNA Polymerase, 5 U/µL	10X DreamTaq Buffer*
#EP0701	200 U	1.25 mL
#EP0702	500 U	2 × 1.25 mL
#EP0703	5 × 500 U	10 × 1.25 mL
#EP0704	20 × 500 U	40 × 1.25 mL
#EP0705	10 × 500 U	20 × 1.25 mL

* includes 20 mM $MgCl_2$

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Description

Thermo Scientific™ DreamTaq™ DNA Polymerase is an enhanced *Taq* DNA polymerase optimized for all standard PCR applications. It ensures higher sensitivity, longer PCR products and higher yields compared to conventional *Taq* DNA polymerase.

DreamTaq DNA Polymerase uses the same reaction set-up and cycling conditions as conventional *Taq* DNA polymerase. Extensive optimization of reaction conditions is not required. The enzyme is supplied with optimized DreamTaq buffer, which includes 20 mM $MgCl_2$.

DreamTaq DNA Polymerase generates PCR products with 3'-dA overhangs. The enzyme is inhibited by dUTP but can incorporate modified nucleotides.

Features

- Robust amplification with minimal optimization.
- High yields of PCR products.
- Higher sensitivity compared to conventional *Taq* DNA polymerase.
- Amplification of long targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- Generates 3'-dA overhangs.
- Incorporates 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.
- 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 min at 74 °C.

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 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 DNA Polymerase in PCR applications. 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 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 µL reaction:

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 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 additional optimization is required, 25 mm $MgCl_2$ (#R0971) can be added to the master mix. The volume of water should be reduced accordingly.

Volumes of 25 mM $MgCl_2$, 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 spin down.
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-5	30 s	
Extension*	72	1 min	
Final Extension	72	5-15 min	1

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

DreamTaq DNA Polymerase does not incorporate dUTP, therefore it is not possible to perform carryover contamination prevention with UDG. For this application we recommend using *Taq* DNA Polymerase (#EP0401) or Thermo Scientific™ DreamTaq™ Hot Start DNA polymerase (#EP1701).

GUIDELINES FOR PRIMER DESIGN

Use primer design software or follow the 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.

- When introducing restriction enzyme sites into primers, refer to the table "Reaction conditions for FastDigest enzymes" located on www.thermofisher.com/fastdigest 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 for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation 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 Thermo Scientific™ 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 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. The $MgCl_2$ 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, the 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) present in the reaction mixture.

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, please 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

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

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 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 a longer initial denaturation step is required, or if the DNA is denatured at a higher temperature, DreamTaq 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, 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 in the reaction 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 DreamTaq DNA Polymerase is 70-75°C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb. For amplification of templates >6 kb a reduction of the extension temperature to 68°C is recommended to avoid enzyme inactivation during prolonged extension times.

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 (for instance, using Thermo Scientific™ InsTAclone™ PCR Cloning Kit (#K1213)), the final extension step may be prolonged to 30 min 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 please visit www.thermofisher.com

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No conversion of covalently closed circular DNA to nicked DNA was detected.

Exodeoxyribonuclease Assay

No degradation of DNA was observed after incubation of DNA fragments with DreamTaq DNA Polymerase.

Ribonuclease Assay

No contaminating RNase activity was detected

Functional Assay

DreamTaq DNA Polymerase was tested for amplification of 956 bp single copy gene from human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene