PRODUCT INFORMATION
Thermo Scientific
DreamTaq DNA Polymerase

#EP0703 5 x 500 U
Lot: ___  Expiry Date: ___
Store at -20°C

Ordering Information

<table>
<thead>
<tr>
<th>Component</th>
<th>DreamTaq DNA Polymerase, 5 U/µL</th>
<th>10X DreamTaq Buffer*</th>
</tr>
</thead>
<tbody>
<tr>
<td>#EP0701</td>
<td>200 U</td>
<td>1.25 mL</td>
</tr>
<tr>
<td>#EP0702</td>
<td>500 U</td>
<td>2 x 1.25 mL</td>
</tr>
<tr>
<td>#EP0703</td>
<td>5 x 500 U</td>
<td>10 x 1.25 mL</td>
</tr>
<tr>
<td>#EP0704</td>
<td>20 x 500 U</td>
<td>40 x 1.25 mL</td>
</tr>
<tr>
<td>#EP0705</td>
<td>10 x 500 U</td>
<td>20 x 1.25 mL</td>
</tr>
</tbody>
</table>

* Includes 20 mM MgCl2.

www.thermoscientific.com/onebio

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 setup 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 MgCl2. 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 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 deoxynucleotides into a polydeoxynucleotide 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 MgCl2, 1 mM 2-mercaptoethanol, 50 mM NaCl, 0.1 mg/mL BSA, 0.75 mM activated calf thymus DNA, 0.2 mM of each dNTP, 0.4 MBq/mL [3H]-dDTTP.

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 (NH4)2SO4 at a ratio optimized for robust performance of DreamTaq DNA Polymerase in PCR applications. DreamTaq Buffer also includes MgCl2 at a concentration of 20 mM.

Inhibition and Inactivation
- Inhibitors: ionic detergents (deoxycholate, sarcosyl 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:

<table>
<thead>
<tr>
<th>10X DreamTaq Buffer*</th>
<th>5 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>dNTP Mix, 2 mM each (#R0241)</td>
<td>5 µL (0.2 mM of each)</td>
</tr>
<tr>
<td>Forward primer</td>
<td>0.1-1.0 µM</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>0.1-1.0 µM</td>
</tr>
<tr>
<td>Template DNA</td>
<td>10 pg - 1 µg</td>
</tr>
<tr>
<td>Water, nuclease-free (#R0581)</td>
<td>to 50 µL</td>
</tr>
<tr>
<td>Total volume</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

*10X DreamTaq Buffer contains 20 mM MgCl2, which is optimal for most applications. If additional optimization is required, 25 mm MgCl2 (#R0971) can be added to the master mix. The concentration of water should be reduced accordingly.

Volumes of 25 mM MgCl2 required for specific final MgCl2 concentration:

- Final concentration of MgCl2: 2 mM
- Volume of 25 mM MgCl2 to be added for 50 µL reaction: 0 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:

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature, °C</th>
<th>Time</th>
<th>Number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95</td>
<td>1-3 min</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>30 s</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>Tm-5</td>
<td>30 s</td>
<td>25-40</td>
</tr>
<tr>
<td>Extension*</td>
<td>72</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>72</td>
<td>5-15 min</td>
<td>1</td>
</tr>
</tbody>
</table>

* 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 PRIMER DESIGN
Use the Thermo Scientific REviewer primer design software at www.thermoscientific.com/reviewer 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 complementarity 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 “Cleaveage efficiency close to the termini of PCR fragments” located on www.oneligo.com 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 e.g., REviewer (www.thermoscientific.com/reviewer) is 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 GeneJET Plasmid Miniprep Kit (#K0532). 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**
DreamTaq DNA Polymerase is provided with an optimized 10X DreamTaq Buffer which includes MgCl₂ at a concentration of 20 mM. A final MgCl₂ concentration of 2 mM is generally ideal for PCR. The MgCl₂ concentration can be further increased up to 4 mM by the addition of 25 mM MgCl₂ (#R0971). 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 1 Mg²⁺).

**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:

<table>
<thead>
<tr>
<th>Volume of PCR mixture</th>
<th>dNTP Mix, 2 mM each (#R0421)</th>
<th>dNTP Mix, 10 mM each (#R0191)</th>
<th>dNTP Mix, 25 mM each (#R1121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 µL</td>
<td>5 µL</td>
<td>1 µL</td>
<td>0.4 µL</td>
</tr>
<tr>
<td>25 µL</td>
<td>2.5 µL</td>
<td>0.5 µL</td>
<td>0.2 µL</td>
</tr>
<tr>
<td>20 µL</td>
<td>2 µL</td>
<td>0.4 µL</td>
<td>0.16 µL</td>
</tr>
</tbody>
</table>

**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 1.15 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 (#K1213), the final extension step can be omitted.

**Troubleshooting**
For troubleshooting please visit www.thermoscientific.com/onebio

**CERTIFICATE OF ANALYSIS**

**Endodeoxyribonuclease Assay**
No conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 10 units of DreamTaq DNA Polymerase with 1 µg of puC19 DNA for 4 hours at 37°C.

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

**Ribonuclease Assay**
No contaminating RNase activity was detected after incubation of 10 units of DreamTaq DNA Polymerase with 1 µg of [32P]-RNA for 4 hours at 37°C.

**Functional Assay**
DreamTaq DNA Polymerase was tested for amplification of 665 bp single copy gene from human genomic DNA and for amplification of 20 kb lambda DNA fragment.

**Quality authorized by:**
Jurgita Zilinskiene

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