Thermo Scientific DyNAzyme II DNA Polymerase

F-503S, 250 U, F-503L, 1000 U

Store at -20°C

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1. Introduction

Thermo Scientific DyNAzyme II DNA Polymerase is a thermostable DNA polymerase for routine DNA amplification. It is purified from an E. coli strain that carries a plasmid encoding DyNAzyme™ DNA polymerase gene from Thermus brockianus. DyNAzyme II DNA polymerase possesses the following activities: 5´→3´ DNA polymerase activity and 5´→3´ exonuclease activity. DyNAzyme II DNA Polymerase lacks the 3´→5´ proofreading activity.

2. Package information

F-503S	250 U (2 U/µl) Material provided: DyNAzyme II DNA Polymerase 250 U (125 µl), 10x Mg^{2+}-free DyNAzyme Buffer (2 x 1.5 ml), 50 mM MgCl2 solution (1 x 1.5 ml).
F-503L	1000 U (2 U/µl) Material provided: DyNAzyme I DNA Polymerase 1000 U (500 µl), 10x Mg^{2+}-free DyNAzyme Buffer (4 x 1.5 ml), 50 mM MgCl2 solution (2 x 1.5 ml).

Material safety datasheet (MSDS) is available at www.thermoscientific.com/fzmsds.

3. Guidelines for using DyNAzyme II DNA Polymerase

For standard PCR reactions, the optimal Mg^{2+} concentration is usually 0.5–1 mM above the total dNTP concentration. We recommend using 1.5 mM Mg^{2+} as an initial concentration. However, some applications may require different Mg^{2+} concentrations. In these cases, optimize the Mg^{2+} concentration using the 50 mM MgCl2 solution provided. See section 4.2 for more information.

Mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. Always pipette DyNAzyme II DNA Polymerase carefully and gently. The high glycerol content (50 %) in the storage buffer may otherwise lead to pipetting errors.

Table 1. Pipetting instructions (add items in this order).

Component	Volume	Final conc.
H_2O	add to 50 µl	
10x Mg^{2+}-free DyNAzyme buffer	5 µl	1x
50 mM $MgCl_2$ solution	1.5 µl*	1.5 mM
10 mM dNTPs	1 µl	200 µM each
Primer A	x µl	0.5 µM**
Primer B	x µl	0.5 µM**
Template DNA	x µl	
DyNAzyme II DNA Polymerase	0.25–1 µl**	0.01-0.04 U/µM (0.5–2 U/50 µl)

* The recommendation for starting Mg^{2+} concentration is 1.5 mM but it can be optimized between 0.75–4 mM, if needed.
** The recommendation for final primer concentration is 0.5 µM but it can be optimized between 0.2–1.0 µM, if needed.
*** Possible enzyme dilutions are recommended to be made in 1x reaction buffer or H_2O immediately before use.

Table 2. Cycling instructions.

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	94°C	1–2	94°C	1–2 min	1
Denaturation Annealing (see 5.2) Extension	94°C – 72°C	15 s–1min – 40 s/kb	94°C Tm -5°C 72°C	15 s –1min 10–30 s 40 s/kb	25–35
Final extension	72°C 4°C	5–10 min hold	72°C 4°C	5–10 min hold	1

4. Notes about reaction components

4.1 Enzyme

The optimal amount of enzyme depends on the amount of the template and the length of the PCR product. Usually 1 unit of DyNAzyme II DNA Polymerase per 50 µl reaction volume gives good results, but the optimal amount can range between 0.5 to 2.0 units per 50 µl reaction depending on the lenght and difficulty of the amplicon.

4.2 Mg^{2+} and dNTP

The concentration of Mg^{2+} is critical since DyNAzyme II DNA Polymerase is a magnesium dependent enzyme. Excessive Mg^{2+} stabilizes the DNA double strand and prevents complete denaturation of DNA. Excess Mg^{2+} can also stabilize spurious annealing of primers to incorrect template sites and decrease specificity. Conversely, inadequate Mg^{2+} may lead to lower product yield. The optimal Mg^{2+} concentration also depends on the dNTP concentration, the specific template DNA and the sample buffer composition. In general, the optimal Mg^{2+} concentration is 1.5 mM. If the primers and/or template contain chelators such as EDTA or EGTA, the apparent Mg^{2+} optimum may be shifted to higher concentrations. On the other hand, some applications may require lower Mg^{2+} concentrations. Therefore we recommend optimizing the Mg^{2+} concentration between 0.75 and 4.0 mM when needed.

High quality dNTPs should be used for optimal performance with DyNAzyme II DNA Polymerase. The polymerase also incorporates nucleotide analogs such as dUTP, dITP and fluorescently-labeled nucleotides.

4.3 Template

General guidelines for low complexity DNA (e.g. plasmid, lambda or BAC DNA) are: 1 pg–10 ng per 50 µl reaction volume. For high complexity genomic DNA, the amount of DNA template should be 50–500 ng per 50 µl reaction volume. If cDNA synthesis reaction mixture is used directly as a source for the template, the volume used should not exceed 10 % of the final PCR reaction volume.

4.4 PCR additives

PCR additives such as DMSO, formamide, glycerol and betaine are compatible with DyNAzyme II DNA Polymerase. We recommend using PCR additives in the following concentrations: DMSO 2–10 %, formamide 2–10 %, glycerol 5–10 %, or combinations of these. Recommended starting point is 5 % DMSO.

Note: If high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. It has been reported that 10 % DMSO decreases the annealing temperature by 5.5–6.0°C.1

5. Notes about cycling conditions

5.1 Denaturation

After an initial 1–2 min denaturation at 94°C, keep the denaturation time as short as possible (usually 30 seconds or less at 94°C). **Note:** The denaturation time and temperature also depend on the ramp rate and temperature control mode of the cycler.

5.2 Primer annealing

The Tm's should be calculated with the nearest neighbor method[2] as results from primer Tm calculations can vary significantly depending on the method used. Instructions for Tm calculation and a link to a calculator using a modified nearest-neighbor method can be found on www.thermoscientific.com/pcrwebtools. We suggest the primers to be annealed for 30 seconds or less at the highest temperature that will permit annealing of the primers to the template. A guideline for DyNAzyme II DNA Polymerase is to use annealing temperature 5°C below the lower Tm of the primers. Two-step cycling without an annealing step is recommended for high Tm primer pairs.

5.3 Extension

The extension for standard PCR should be performed at 72°C (40 seconds per one kilobase of amplified product).

6. Component specifications

6.1 DyNAzyme II DNA Polymerase (F-501)

The thermostable DyNAzyme II DNA Polymerase is purified from an E. coli strain expressing the cloned DyNAzyme DNA Polymerase gene from Thermus brockianus, Thermo Scientific proprietary bacterial strain. DyNAzyme II DNA Polymerase is free of contaminating endo- and exonucleases. It has a half life of 2.5 h at 96°C.

Storage buffer: 20 mM Tris-HCl (pH 7.4 at 25°C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers, 200 µg/ml BSA and 50 % glycerol.

Unit definition: One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTPs into acid-insoluble form at 74°C in 30 minutes under the stated assay conditions.

Unit assay conditions: Incubation buffer: 25 mM TAPS-HCl, pH 9.3 (at 25°C), 50 mM KCl, 2 mM MgCl2,1 mM β-mercaptoethanol, 100 µM dCTP, 200 µM each dATP, dGTP, dTTP.

Incubation procedure: 20 µg activated calf thymus DNA and 0.5 µCi [β-32P] dCTP are incubated with 0.1 units DNA polymerase in 50 µl incubation buffer at 74°C for 10 minutes. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

DNA amplification assay: Performance in PCR is tested by the amplification of a 500 bp fragment of lambda DNA and a 6 kb fragment of M13 DNA.

Exonuclease contamination assay: Incubation of 10 U for 4 hours at 72°C in 50 µl assay buffer with 1 µg sonicated [3H] ssDNA (2 x 105 cpm/µg) released <1 % of radioactivity.

Endonuclease contamination assay: No endonuclease activity is observed after incubation of 10 U of DNA polymerase with 1 µg of lambda DNA or lambda HindIII DNA fragments in assay buffer at 72°C for 4 hours.

6.2 10x Mg^{2+}-free DyNAzyme buffer (F-510)

In final 1x concentration the Mg^{2+}-free DyNAzyme buffer contains 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl and 0.1 % Triton® X-100.

6.3 50 mM $MgCl_2$ solution (F-510MG)

The 50 mM MgCl2 solution can be used with the Mg^{2+}-free DyNAzyme buffer.

7. References

1. Chester N. & Marshak D.R. (1993) Analytical Biochemistry 209: 284–290.

2. Breslauer K.J. et al., (1986) PNAS 83: 3746–3750.

Shipping and storage

Phusion DNA Polymerase is shipped on gel ice. Upon arrival, store the components at -20°C.

Technical support:

US: techservice.genomics@thermofisher.com
Europe, Asia, Rest of World:
techservice.emea.genomics@thermofisher.com

Web: www.thermoscientific.com/phusion
Tm-calculator: www.thermoscientific.com/pcrwebtools

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