

## Thermo Scientific DyNAzyme I DNA Polymerase, Native Enzyme

F-500S/L, 100 U / 500 U

Store at -20°C



### 1. Introduction

Thermo Scientific DyNAzyme I is a thermostable DNA polymerase. It is isolated and purified from *Thermus brockianus* (Thermo Scientific's proprietary strain). DyNAzyme™ I DNA Polymerase has a half life of 3 hours at 96°C. DyNAzyme I DNA Polymerase possesses the following activities: 5'→3' DNA polymerase activity and 5'→3' exonuclease activity. DyNAzyme I DNA Polymerase lacks the 3'→5' proofreading activity. DyNAzyme I DNA Polymerase is purified free of contaminating endo- and exonucleases.

### 2. Package information

F-500S	F-500S: 100 U (2U/μl) Material provided: DyNAzyme I DNA Polymerase 100 U (50 μl) Optimized DyNAzyme buffer (1.5 ml)
F-500L	F-500L: 500 U (2U/μl) Material provided: DyNAzyme I DNA Polymerase 500 U (250 μl) Optimized DyNAzyme buffer (2 x 1.5ml)

Material safety data sheet (MSDS) is available at [www.thermoscientific.com/fzmsds](http://www.thermoscientific.com/fzmsds)

### 3. Guidelines for using DyNAzyme I DNA Polymerase

#### 3.1. Basic reaction conditions for DNA amplification

For standard PCR reactions, optimized DyNAzyme buffer is recommended (1x buffer contains 1.5 mM Mg<sup>2+</sup>). In case lower Mg<sup>2+</sup> concentrations are required, use the Mg<sup>2+</sup>-free DyNAzyme buffer with 50 mM MgCl<sub>2</sub> 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 I 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 <sub>2</sub> O	add to 50 μl	
10x Optimized DyNAzyme buffer	5 μl	1x (1.5 mM MgCl <sub>2</sub> )
10 mM dNTP mix	1 μl	200 μM each
primer A*	x μl	0.5 μM
primer B*	x μl	0.5 μM
template DNA	x μl	
DyNAzyme I DNA Polymerase	0.25–1 μl**	0.01–0.04 U/μl (0.5–2 U/50 μl)

\* The recommendation for final primer concentration is 0.5 μM but it can be varied in a range of 0.2–1.0 μM, if needed.

\*\* Possible enzyme dilutions are recommended to be made in 1x reaction buffer or H<sub>2</sub>O 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 min	94°C	1 – 2 min	1
Denaturation	94°C	15 s – 1 min	94°C	15 s–1 min	25–35
Annealing (see 5.2)	–	–	Tm -5°C	10–30 s	
Extension	72°C	40 s/kb	72°C	40 s/kb	
Final extension	72°C	5–10 min	72°C	5–10 min	1
	4°C	hold	4°C	hold	

### 4. Notes about reaction components

#### 4.1 Enzyme

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

#### 4.2 Mg<sup>2+</sup> and dNTP

The concentration of Mg<sup>2+</sup> is critical since DyNAzyme I DNA Polymerase is a magnesium dependent enzyme. Excessive Mg<sup>2+</sup> stabilizes the DNA double strand and prevents complete denaturation of DNA. Excess Mg<sup>2+</sup> can also stabilize spurious annealing of primers to incorrect template sites and decrease specificity. Conversely, inadequate Mg<sup>2+</sup> may lead to lower product yield. The optimal Mg<sup>2+</sup> concentration also depends on the dNTP concentration, the specific template DNA and the sample buffer composition. If the optimized buffer (1.5 mM Mg<sup>2+</sup> final concentration) does not give satisfactory results, optimize the Mg<sup>2+</sup> concentration between 0.75 and 4.0 mM. In general, the optimal Mg<sup>2+</sup> concentration is 0.5 to 1 mM over the total dNTP concentration for standard PCR. If the primers and/or template contain chelators such as EDTA or EGTA, the apparent Mg<sup>2+</sup> optimum may be shifted to higher concentrations.

High quality dNTPs should be used for optimal performance with DyNAzyme I 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 I 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 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 T<sub>m</sub>'s should be calculated with the nearest-neighbor method<sup>2</sup> as results from primer T<sub>m</sub> calculations can vary significantly depending on the method used. Instructions for T<sub>m</sub> calculation and a link to a calculator using the nearest-neighbor method can be found on [www.thermoscientific.com/pcrwebtools](http://www.thermoscientific.com/pcrwebtools). We suggest the primers to be annealed for one minute or less at the highest temperature that will permit annealing of the primers to the template. A guideline for DyNAzyme I DNA Polymerase is to use an annealing temperature 5°C below the lower T<sub>m</sub> of the primers. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR). Two-step cycling without an annealing step is also recommended for high T<sub>m</sub> primer pairs.

#### 5.3 Extension

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

### 6. Component specifications

#### 6.1 DyNAzyme I DNA Polymerase (F-500)

The native thermostable DyNAzyme I DNA Polymerase is purified from *Thermus brockianus*, Thermo Scientific proprietary bacterial strain. DyNAzyme I DNA Polymerase has a half life of 3 h at 96°C. DyNAzyme I DNA Polymerase is free of contaminating endo- and exonucleases.

**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 MgCl<sub>2</sub>, 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 [α-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 500 bp lambda DNA and 6 kb M13 DNA. **Exonuclease contamination assay:** Incubation of 10 U for 4 hours at 72°C in 50 μl assay buffer with 1 μg sonicated [<sup>3</sup>H] 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 Optimized DyNAzyme Buffer (F-511)

In final 1x reaction concentration the Optimized DyNAzyme buffer contains 10 mM Tris-HCl (pH 8.8 at 25°C), 50 mM KCl, 1.5 mM MgCl<sub>2</sub> and 0.1 % Thermo Scientific Triton X-100.

## 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

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

## Technical support

US: [techservice.genomics@thermofisher.com](mailto:techservice.genomics@thermofisher.com)

Europe, Asia, Rest of World:

[techservice.emea.genomics@thermofisher.com](mailto:techservice.emea.genomics@thermofisher.com)

Web: [www.thermoscientific.com/pcr](http://www.thermoscientific.com/pcr)

Tm-calculator: [www.thermoscientific.com/pcrwebtools](http://www.thermoscientific.com/pcrwebtools)

## Product use limitation

This product has been developed and is sold exclusively for research purposes and in vitro use only. This product has not been tested for use in diagnostics or drug development, nor are they suitable for administration to humans or animals.

Designed and manufactured according to certified ISO9001:2008 processes.

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