Thermo Scientific DyNAzyme I DNA Polymerase Kit

F-550S/L, 100 U/500 U

Store at -20°C

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1. Introduction

Thermo Scientific DyNAzyme I DNA Polymerase is a thermostable DNA polymerase for routine DNA amplification. It is isolated and purified from *Thermus brockianus*, Thermo Scientific's proprietary strain. DyNAzyme™ I DNA polymerase possesses 5´→3´ DNA polymerase activity and 5´→3´ exonuclease activity but lacks 3´→5´ proofreading activity. DyNAzyme I DNA Polymerase Kit includes all the necessary reagents for 100 (F-550S) or 500 (F-550L) amplification reactions of 50 µl. It also includes a lambda DNA control template and primers for a 500 bp amplicon. The template amount is sufficient for performing 40 control amplifications.

2. Kit components

Component	Concent.	F-550S	F-550L
DyNAzyme I DNA Polymerase	2 U/µl	100 U	500 U
Optimized DyNAzyme buffer		1 x 1.5 ml	2 x 1.5 ml
Mg^{2+}-free DyNAzyme buffer		1 x 1.5 ml	2 x 1.5 ml
dNTP Mix	10 mM each	100 µl	500 µl
Mg^{2+}-solution	50 mM	1 x 1.5 ml	1 x 1.5 ml
Control lambda template	0.5 ng/µl	40 µl	40 µl
500 bp control primers	25 µM each	40 µl	40 µl
DNA size standard		400 µl	400 µl

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

3. Guidelines for using DyNAzyme I DNA Polymerase

3.1 Basic reaction conditions for DNA amplifications

For standard PCR reactions, optimized DyNAzyme buffer is recommended (1x buffer contains 1.5 mM Mg^{2+}). In case lower Mg^{2+} concentrations are required, use the Mg^{2+}-free DyNAzyme buffer with 50 mM $MgCl_2$ 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 concentration
H_2O	Add to 50 µl	
10x Optimized DyNAzyme buffer	5 µl	1x (1.5 mM $MgCl_2$)
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 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_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 min	94°C	1–2 min	1
Denaturation Annealing (see 5.2) Extension	94°C – 72°C	15 s–1 min – 40 s/kb	94°C Tm -5°C 72°C	15 s–1 min 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 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 lenght and difficulty.

4.2 Mg^{2+} and dNTP

The concentration of Mg^{2+} is critical since DyNAzyme I 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. If the optimized buffer (1.5 mM Mg^{2+} final concentration) does not give satisfactory results, optimize the Mg^{2+} concentration between 0.75 and 4.0 mM. In general, the optimal Mg^{2+} 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^{2+} 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 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 the nearest-neighbor method can be found on website www.thermoscientific.com/pcrwebstools. 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 I DNA Polymerase is to use an annealing temperature 5°C below the lower Tm 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 Tm primer pairs.

5.3 Extension

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

6. Amplifying control template

6.1 Reaction conditions

Table 3. Pipetting instructions for control template.

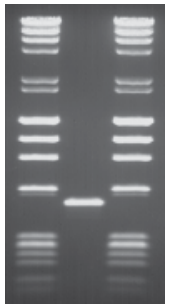
Component	Volume	Final concentration
H_2O	41.5 µl	
10x Optimized DyNAzyme buffer	5 µl	1x (1.5 mM $MgCl_2$)
10 mM dNTPs	1 µl	200 µM each
500 bp control primer mix	1 µl	0.5 µM each
Control template DNA	1 µl	0.5 ng/50 µl
DyNAzyme I DNA Polymerase	0.5 µl	1 U/50 µl

Table 4. Cycling instructions for control template.

Cycle step	Temp.	Time	Cycles
Initial denaturation	94°C	1 min	1
Denaturation Annealing Extension	94°C 60°C 72°C	15 s 30 s 30 s	20
Final extension	72°C	10 min	1

The cycling protocol above is a recommendation. If you wish to run the control together with your experimental samples, please note that the control has been shown to work in a variety of conditions.

6.2 Analysis of the control reaction



In the image on the left the control reaction has been run on an ethidium bromide stained agarose gel (1 %). For this run, 15 µl of the reaction mixture was loaded on the gel.

Lanes 1 and 3: DNA size standard (F-303 SD)

Lane 2: Amplified 500 bp product of control PCR.

After running your control reaction on a gel, compare the results to the image on the left to check for specificity and efficiency of the reaction.

7. Component specifications

7.1 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 $MgCl_2$, 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 [^{3}H] ssDNA (2 x 10^5 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 *Hind*III DNA fragments in assay buffer at 72°C for 4 hours.

7.2 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 $MgCl_2$ and 0.1 % Triton® X-100.

7.3 10x Mg2+-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.

7.4 dNTP Mix (F-560)

The dNTP Mix is ready-to-use solution consisting of the following compounds: dATP, dGTP, dCTP and dTTP dissolved in ddH_2O at 10 mM each.

7.5 50 mM MgCl2 solution (F-510MG)

The 50 mM MgCl2 solution can be used with the Mg^{2+}-free DyNAzyme buffer or to supplement the Mg^{2+} concentration in the Optimized DyNAzyme Buffer.

7.6 Lambda control template (F-304K)

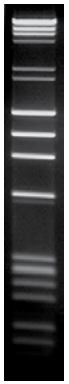
The control template is bacteriophage lambda DNA (GenBank accession number J02459, 48 502 bp). The concentration is 0.5 ng/µl in TE-buffer.

7.7 500 bp control primers (F-557)

The control primer #1 is a 23-mer forward primer. The sequence is 5´-GATGAGTTCGTGTCCGTACAACT-3´ and the melting point is 64.4°C. The primer coordinates are 7131–7153 on the lambda template. The control primer #2 is a 23-mer reverse primer. The sequence is 5´ GGTTATCGAAATCAGC-CACAGCG-3´ and the melting point is 70.5°C. The primer coordinates are 7608–7630 on the lambda template. Each primer concentration is 25 µM.

7.8 Ready-to-use DNA size standard (F-303SD)

The DNA standard is a mix of lambda DNA *Hind*III digest and bacteriophage ΦX174 DNA HaeIII digest, each at 50 ng/µl (100 ng/µl total). It is supplied in 8 mM Tris-HCl (pH 8.0), 12 mM EDTA, 12 % glycerol and 0.012 % (w/v) bromophenol blue dye. The DNA standard solution contains 19 fragments of the following sizes and mass amounts (per 10 µl):



Fragment	Base pairs	DNA amount ng/10 µl
1	23 130	238
2	9 416	97
3	6 557	68
4	4 361	45
5	2 322	24
6	2 027	21
7	1 353	126
8	1 078	100
9	872	81
10	603	56
11	*564	6
12	310	29
13a	281	26
13b	271	25
14	234	22
15	194	18
16a	125	1
16b	118	11
17	72	7

* Due to the low amount of DNA these bands are almost invisible.

8. 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 Kit 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/pcr
Tm-calculator: 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.

v1_07.2011

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