

Thermo Scientific DyNAzyme II DNA Polymerase Kit

F-551S/L, 100 U/500 U

Store at -20°C



1. Introduction

Thermo Scientific DyNAzyme II DNA Polymerase is a thermostable DNA polymerase for routine DNA amplification in the laboratory. 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 5'→3' DNA polymerase activity and 5'→3' exonuclease activity but lacks 3'→5' proofreading activity.

DyNAzyme II DNA Polymerase Kit includes all necessary reagents for 100 (F-551S) or 500 (F-551L) amplification reactions of 50 µl. It also includes 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	Conc.	F-551S	F-551L
DyNAzyme II 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 II DNA Polymerase

3.1. Basic reaction conditions for PCR

For standard PCR reactions, optimized DyNAzyme buffer is recommended (1x buffer contains 1.5 mM Mg²⁺). In case lower Mg²⁺ concentrations are required, use the Mg²⁺-free DyNAzyme buffer with 50 mM MgCl₂ solution provided. See section 5.2 for more information.

Mix and centrifuge all tubes before opening to improve recovery. Pipette DyNAzyme II DNA Polymerase always 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 ₂ O	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 II 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 optimized between 0.2-1.0 µM, if needed.

** Possible enzyme dilutions are recommended to be made in 1x reaction buffer or H₂O 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 6.2)	–	–	T _m - 5°C	10-30 s	
Extension	72°C	40 s/1 kb	72°C	40 s/1 kb	
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 the enzyme depends on the amount of the template and the length of the PCR product. Usually 1 unit of the DyNAzyme II DNA Polymerase per 50 µl reaction gives good results, but optimal amounts can range between 0.5-2.0 units per 50 µl reaction.

4.2 Mg²⁺ concentration and dNTP concentration

Optimization of Mg²⁺ is critical since DyNAzyme II DNA Polymerase is a magnesium dependent enzyme. In addition to DyNAzyme II DNA Polymerase, the template DNA, primers, and dNTPs bind Mg²⁺.

Therefore, the optimal Mg²⁺ concentration will depend on the dNTP concentration, the specific template DNA, and the sample buffer composition.

If the optimized buffer (1.5 mM Mg²⁺ final concentration) does not give satisfactory results, optimize the Mg²⁺ concentration between 0.75 and 4.0 mM. Excessive Mg²⁺ stabilizes the DNA double strand and prevents complete denaturation of DNA, which reduces the yield. Excess Mg²⁺ can also stabilize spurious annealing of primer to incorrect template sites, decreasing specificity. Conversely, inadequate Mg²⁺ may lead to lower product yield. In general, the acceptable Mg²⁺ concentration range narrows as the length of the amplicon increases. Usually the optimal Mg²⁺ concentration is 0.5 to 1 mM above the total dNTP concentration for standard PCR. **Note:** If the primers and/or template contain chelators such as EDTA or EGTA, the apparent Mg²⁺ optimum may be shifted.

High quality dNTPs should be used for optimal performance with DyNAzyme II DNA Polymerase.

4.3 Incorporation of nucleotide analogs

DyNAzyme II DNA Polymerase incorporates dUTP, dITP and fluorescently-labeled nucleotides.

4.4 Template

General guidelines are: 1 pg - 10 ng/50 µl reaction with low complexity genomes (e.g. plasmid, lambda or BAC DNA); 50-500 ng/50 µl reaction with high complexity genomic DNA.

4.5 PCR additives

DyNAzyme II DNA Polymerase tolerates high DMSO concentrations (up to 10 %) needed for opening of difficult templates. Also other additives which help DNA denaturation can be used with DyNAzyme II DNA Polymerase (formamide, glycerol, betaine, or combinations of these). We recommend using PCR additives in the following concentrations: DMSO 2-10 %, Formamide 2-10 %, Glycerol 5-10 %. Recommended starting point is 5 % DMSO. **Note:** In high DMSO concentrations the annealing temperature must be lowered, because DMSO decreases the melting point of the primers. For example, 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 cyler.

5.2 Primer annealing

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 determination of the annealing temperature is to use a temperature 5°C lower than the lower T_m calculated by the nearest-neighbor method.² 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 annealing step is recommended also for high T_m primer pairs. Instructions for T_m

calculation and a link to a calculator using the nearest-neighbor method can be found on www.thermoscientific.com/pcrwebtools.

5.3 Extension

The extension for standard PCR 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 conc.
H ₂ O	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 II DNA Polymerase	0.5 µl	1 U/50 µl

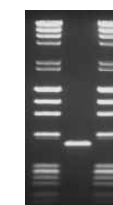
6.2 Cycle conditions

Table 4. Cycling instructions for control template.

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

6.3 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 in 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 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's proprietary bacterial strain. DyNAzyme II DNA Polymerase has a half life of 2.5 h at 96°C. DyNAzyme II DNA Polymerase is purified 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 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 [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 [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 *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 MgCl₂ and 0.1 % Triton® X-100.

7.3 10x Mg²⁺-free DyNAzyme buffer (F-510)

In final 1x concentration the Mg²⁺-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 H₂O at 10 mM each.

7.5 50 mM MgCl₂ solution (F-510MG)

The 50 mM MgCl₂ solution can be used with the Mg²⁺-free DyNAzyme buffer or to supplement the Mg²⁺ 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 upper primer. The sequence is 5'-GATGAGTTCGTCCGTACAAC-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 lower primer. The sequence is 5'-GGTTATCGAAATCAGCCA-CAGCG-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 standard (F-303SD)

The DNA standard is a mix of lambda DNA *Hind*III digest and bacteriophage φX174 DNA *Hae*III 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

Note: The cohesive areas of fragments 1 and 4 can be separated by heating at 65°C for 5 minutes. For daily use the marker can be stored at +4°C (at least one month). The marker is stable at -20°C for at least one year.

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

8. References

1. Chester & Marshak (1993) *Analytical Biochemistry* 209, 284-290.
2. Breslauer *et al.* (1986) *PNAS* 83, 3746-3750.

Storage and shipping

DyNAzyme II 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.

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