Thermo Scientific DyNAzyme EXT DNA Polymerase

F-505S/L, 200 U/1000 U

Store at -20°C

1. Introduction

Thermo Scientific DyNAzyme EXT DNA Polymerase is a versatile and easy to use enzyme, with powerful advantages for all PCR applications. It is especially suitable for difficult templates and long PCR. DyNAzyme™ EXT DNA Polymerase is an optimized mixture of DyNAzyme II DNA Polymerase and a proofreading enzyme. The DNA polymerase activity of DyNAzyme II DNA Polymerase provides the highly efficient polymerization needed for successful long and difficult PCR and also for high yields in standard PCR. The proofreading activity removes misincorporated nucleotides, which would otherwise either be extended and thus produce mutations, or block further chain extension. DyNAzyme EXT DNA Polymerase possesses the following activities: 5'→3' DNA polymerase activity, 5'→3' exonuclease activity, and a weak 3'→5' exonuclease activity. It is capable of adding a non-templated adenine residue at the 3' end of a DNA fragment.

2. Package information

F-505S	200 U (2 U/µl) Material provided: DyNAzyme EXT DNA Polymerase 200 U (200 µl), 10x Optimized DyNAzyme EXT Buffer* (2 x 1.5 ml), 10x Mg^{2+}-free DyNAzyme EXT Buffer (2 x 1.5 ml), 50 mM $MgCl_2$ solution (1.5 ml) and DMSO (500 µl).
F-505L	1000 U (2 U/µl) Material provided: DyNAzyme EXT DNA Polymerase 1000 U (2 x 500 µl), 10x Optimized DyNAzyme EXT Buffer* (4 x 1.5 ml), 10x Mg^{2+}-free DyNAzyme EXT Buffer (4 x 1.5 ml), 50 mM $MgCl_2$ solution (2 x 1.5 ml) and DMSO (500 µl).

* 10x Optimized DyNAzyme EXT Buffer provides 1.5 mM $MgCl_2$ in final reaction concentration.

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

3. Guidelines for using DyNAzyme EXT DNA Polymerase

3.1 Standard and long PCR

Optimized DyNAzyme EXT Buffer (1x buffer contains 1.5 mM Mg^{2+}) is recommended for standard and long PCR reactions up to 10 kb. Some applications may require lower Mg^{2+} concentrations. In these cases, use the Mg^{2+}-free DyNAzyme EXT Buffer, and optimize the Mg^{2+} concentration with the 50 mM $MgCl_2$ solution provided. For optimization of especially long extension reactions, the magnesium concentration in either buffer can be supplemented using the 50 mM $MgCl_2$ solution provided. See section 5.2 for more information.

3.2 Difficult templates

Often the basic reaction conditions are sufficient for successful amplification of difficult templates, such as GC-rich and looped sequences. With the most difficult PCR reactions, improved results can be achieved by using DyNAzyme EXT DNA Polymerase together with a PCR additive, e.g. DMSO, formamide, glycerol, or betaine, which relax DNA, thus making template denaturation easier. A recommended starting point is 5 % DMSO. See section 5.4 for more information on PCR additives.

3.3 Cloning

Primarily, we recommend Phusion® DNA Polymerases for cloning due to their extreme fidelity, but the robustness of DyNAzyme EXT DNA Polymerase and its improved fidelity compared to standard PCR enzymes allows it to be used for cloning applications as well. DyNAzyme EXT DNA Polymerase is capable of adding a non-templated adenine residue at the 3' end of a DNA fragment. PCR products produced with DyNAzyme EXT DNA Polymerase can be used in both TA cloning and blunt cloning.

4. Basic reaction conditions for DNA amplification

Mix and centrifuge all tubes before opening to ensure homogeneity and to improve recovery. Always pipette DyNAzyme EXT DNA Polymerase carefully and gently. The high glycerol content (50 %) in the storage buffer may otherwise lead to pipetting errors. The optimal reaction conditions with DyNAzyme EXT DNA Polymerase vary depending on the length and complexity of your starting template. See the pipetting instructions in Table 1. Long PCR is very sensitive even to small variations in the reaction conditions. Therefore, the optimal conditions need to be determined experimentally.

Useful tips for long PCR:

- Use UTW® (ultra-thin wall) or standard thin-walled tubes.
- Perform a manual hot start or place the reaction mixtures to a pre-heated cycler block directly from ice.

Table 1. Pipetting instructions for amplicons of different size and complexity.

Low complexity DNA (e.g. λ DNA)	<10 kb		10–20 kb		>20 kb	
High complexity DNA (e.g. human genomic DNA)	<7 kb		7–12 kb		> 12 kb	
Component	Volume	Final conc.	Volume	Final conc.	Volume	Final conc.
H_2O	Add to 50 µl		Add to 50 µl		Add to 50 µl	
10x Optimized DyNAzyme EXT Buffer	5 µl	1x (1.5 mM $MgCl_2$)	–	–	–	–
10x Mg-free buffer	–	–	5 µl	1x	5 µl	1x
50 mM $MgCl_2$	–	1.5 mM	1.7 µl	1.7 mM	2.3 µl	2.3 mM
10 mM dNTPs	1 µl	200 µM each	1.8 µl	360 µM each	2.5 µl	500 µM each
Primer A	X µl	0.5 µM*	X µl	0.5 µM*	X µl	0.5 µM*
Primer B	X µl	0.5 µM*	X µl	0.5 µM*	X µl	0.5 µM*
Template DNA (see 5.3)	X µl		X µl		X µl	
(DMSO, optional) (see 5.4)	2.5 µl	(5 %)	(2.5 µl)	(5 %)	(2.5 µl)	(5 %)
DyNAzyme EXT DNA Polymerase (see 5.1)	0.5–3 µl	0.5–3 U	0.5–3 µl	0.5–3 U	0.5–3 µl	0.5–3 U

* The recommended final concentration is 0.5 µM, but it can be optimized in a range of 0.2–1.0 µM, if needed.

Table 2. Cycling instructions for fragments < 10 kb.

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 (see 6.1) Annealing (see 6.2) Extension (see 6.3)	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

Table 3. Cycling instructions for fragments ≥10 kb.

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 (see 6.1) Annealing (see 6.2) Extension (see 6.3)	94°C – 70°C	15 s–1 min – 40 s/kb	94°C Tm - 5°C 70°C	15 s–1 min 15 s–1 min 40 s/kb	10
Denaturation (see 6.1) Annealing (see 6.2) Extension (see 6.3)	94°C – 70°C	15 s–1 min – 40 s/kb + 20 s/cycle	94°C Tm - 5°C 70°C	15 s–1 min 15 s–1 min 40 s/kb + 20 s /cycle	15–20
Final extension	70°C 4°C	5–10 min hold	70°C 4°C	5–10 min hold	1

5. Notes about reaction components

5.1 Enzyme

The optimal amount of enzyme depends on the amount of template and the length of the PCR product. Usually 1 unit of DyNAzyme EXT DNA Polymerase per 50 µl reaction volume gives good results, but for difficult templates and long PCR the optimal amount can vary between 0.5–3 U per 50 µl reaction.

5.2 Mg^{2+} and dNTP

Optimization of Mg^{2+} is critical since DyNAzyme EXT DNA Polymerase is a magnesium dependent enzyme. In addition to the DNA polymerase, also the template DNA, primers and dNTPs bind Mg^{2+}. Therefore, the optimal Mg^{2+} concentration depends on the dNTP concentration, the specific template DNA and the sample buffer composition. Excessive Mg^{2+} stabilizes the DNA double strand and prevents complete denaturation of the DNA, thus reducing yield. Excess Mg^{2+} can also stabilize spurious annealing of primers to incorrect template sites, decreasing specificity. On the other hand, inadequate Mg^{2+} reduces the amount of the PCR product. In general, the optimal Mg^{2+} concentration range narrows as the length of the PCR product increases. For standard PCR the optimal Mg^{2+} concentration is usually 0.5–1 mM over the total dNTP concentration, and for long PCR 0.1–0.5 mM over the total dNTP concentration. High quality dNTPs should be used for optimal performance with DyNAzyme EXT DNA Polymerase. The polymerase cannot read dUTP-derivatives or dITP in the template strand, so the use of these analogues or primers containing them is not recommended.

5.3 Template

Template preparation becomes particularly important when performing long PCR. The amount of template required depends on the length of the PCR product. For longer extensions, more template is needed. 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.

5.4 PCR additives

PCR additives such as DMSO, formamide, glycerol and betaine are compatible with DyNAzyme EXT 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]

6. Notes about cycling conditions

6.1 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). This is particularly important for long PCR. **Note:** The denaturation time and temperature also depend on the ramp rate and the temperature control mode of the cycler.

6.2 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 Tm calculated by the nearest-neighbor method.[2] For long PCR, the primers should be designed to allow a high annealing temperature for maximal reaction specificity (preferably ≥ 65°C). If the Tm's of the primers are high enough, annealing and extension can be performed in a single 70–72°C step (two-step PCR). Instructions for Tm calculation and a link to a calculator using a modified nearest-neighbor method can be found on website www.thermoscientific.com/pcrwebtools.

6.3 Extension

The extension for standard PCR should be performed at 72°C, and for long PCR at 68–70°C. For amplification of shorter DNA fragments (<10 kb), a constant extension time can be used (40 seconds per one kilobase). For amplification of long DNA fragments (≥10 kb), use a constant extension time (40 seconds per one kilobase) for the first 10 cycles. Then, during the next 15–20 cycles, add 20 seconds to the elongation time on each cycle.

7. Troubleshooting

No product at all or low yield	
• Repeat and make sure that there are no pipetting errors. • Use fresh high quality dNTPs. • Do not use dNTP mix or primers that contain dUTP or dITP. • Titrate template amount. • Template DNA may be damaged. Use carefully purified template. • Increase extension time. • Increase the number of cycles. • Decrease annealing temperature.	• Use more enzyme. • Optimize magnesium concentration. • Try adding 2–10 % DMSO in the reaction. • Titrate DMSO (2–8 %) in the reaction (see section 4.5). • Optimize denaturation temperature. • Optimize the denaturation time. • Check the purity and concentration of the primers. • Check primer design.
Non-specific products	
• Increase annealing temperature • Use manual hot start or place reaction mixtures to a pre-heated cycler block directly from ice.	• Decrease primer concentration. • Design new primers. • Optimize Magnesium concentration.
Non-specific products - Low molecular weight discrete bands	
• Decrease magnesium concentration, e.g. using 0.2 mM steps. • Use less enzyme.	• Increase dNTP concentration. • Shorten extension time. • Reduce the number of cycles.

8. Component specifications

8.1 DyNAzyme EXT DNA Polymerase (F-505)

DyNAzyme EXT DNA Polymerase is an optimized mixture of DyNAzyme II DNA Polymerase and a proofreading enzyme. DyNAzyme II DNA Polymerase is purified from an *E.coli* strain expressing the cloned DyNAzyme DNA Polymerase gene from *Thermus brockianus*, a Thermo scientific's proprietary bacterial strain. DyNAzyme EXT DNA Polymerase is free of contaminating endo- and exonucleases. It has a half life of 3.5 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 acidinsoluble form at 74°C in 30 minutes under the stated assay conditions.

Unit assay conditions: Incubation buffer: 25 mM TAPSHCl, pH 9.3 (at 25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 100 µM dCTP, 200 µM each of dATP, dGTP, dTTP. Incubation procedure: 20 µg activated calf thymus DNA and 0.5 µCi [α-^{32}P] dCTP are incubated with 0.1 units of DNA polymerase in 50 µl incubation buffer at 74°C for 10 minutes. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Exonuclease contamination assay: Incubation of 10 U for 4 hours at 72°C in 50 µl assay buffer with 1 µg sonicated [^{3}H] labeled ssDNA ($2x10^5$ cpm/µg) released < 1 % of radioactivity.

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

DNA amplification assay: Performance in PCR is tested by the amplification of 20 kb and 30 kb fragments of lambda DNA.

8.2 10x Optimized DyNAzyme EXT Buffer (F-514)

In final 1x reaction concentration the Optimized DyNAzyme EXT Buffer contains 50 mM Tris-HCl (pH 9.0 at 25°C), 1.5 mM $MgCl_2$, 15 mM $(NH_4)_2SO4$ and 0.1 % Triton® X-100.

8.3 10x Mg^{2+}-free DyNAzyme EXT Buffer (F-512)

In final 1x reaction concentration the Mg^{2+}-free DyNAzyme EXT Buffer contains 50 mM Tris-HCl (pH 9.0 at 25°C), 15 mM $(NH_4)_2SO_4$ and 0.1 % Triton® X-100.

8.4 50 mM $MgCl_2$ Solution (F-510MG)

The 50 mM $MgCl_2$ solution can be used with the Mg^{2+}- free DyNAzyme EXT Buffer or to supplement the Mg^{2+} concentration in the Optimized DyNAzyme EXT Buffer.

8.5 Dimethyl sulfoxide DMSO, 100 % (F-515)

Note: The freezing point of DMSO is 18–19°C, so it does not melt on ice.

9. 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 EXT 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/pcr
Tm-calculator: www.thermoscientific.com/pcrwebtools

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