

## Thermo Scientific DyNAzyme II Hot Start DNA Polymerase

F-504S/L, 250 U/1000 U

Store at -20°C



### 1. Introduction

Thermo Scientific DyNAzyme II Hot Start DNA Polymerase is a modified form of the Thermo Scientific DyNAzyme II DNA Polymerase (F-501) originating from *Thermus brockianus*. DyNAzyme™ II Hot Start DNA Polymerase is inactive at room temperature; the activity is recovered by a 10-minute incubation step at 94°C. The enzyme improves the specificity of PCR by preventing the amplification of non-specific products during reaction setup and the first heating cycle. The use of DyNAzyme II Hot Start DNA Polymerase is also beneficial when using primers that are prone to primer-dimer formation or when using robotic PCR systems that include incubations at room temperature prior to temperature cycling. DyNAzyme II Hot Start DNA Polymerase possesses both 5'→3' DNA polymerase and 5'→3' exonuclease activities.

- Use 10 min initial denaturation to activate DyNAzyme II Hot Start DNA Polymerase.
- Use 1.2 U of DyNAzyme II Hot Start DNA Polymerase per 50 µl reaction volume.

- Note: DyNAzyme II Hot Start DNA Polymerase is provided as a 2 U/µl solution.

**IMPORTANT NOTES**

### 2. Package information

F-504S	250 U (2 U/µl) Material provided: DyNAzyme II Hot Start DNA Polymerase 250 U (125 µl), DyNAzyme II Hot Start Reaction Buffer (1.5 ml).
F-504L	1000 U (2 U/µl) Material provided: DyNAzyme II Hot Start DNA Polymerase 1000 U (500 µl), DyNAzyme II Hot Start Reaction Buffer (4 x 1.5 ml).

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

### 3. Guidelines for using DyNAzyme II Hot Start DNA Polymerase

DyNAzyme II Hot Start DNA Polymerase (2 U/µl) is provided with 10x DyNAzyme II Hot Start Reaction Buffer.

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

When using DyNAzyme II Hot Start DNA Polymerase, it is not necessary to perform PCR setup on ice.

Table 1. Pipetting instructions: add items in this order.

Component	50 µl reaction	20 µl reaction	Final conc.
H <sub>2</sub> O	add to 50 µl	add to 20 µl	
10x DyNAzyme II Hot Start Reaction Buffer*	5 µl	2 µl	1x
10 mM dNTP mix	1 µl	0.4 µl	200 µM each
primer A	x µl	x µl	0.5** µM
primer B	x µl	x µl	0.5** µM
template DNA	x µl	x µl	
DyNAzyme II Hot Start DNA Polymerase	0.6 µl	0.24 µl	0.024 U/ µl

\* Provides 2.5 mM Mg<sup>2+</sup> in 1x reaction.

\*\* The recommendation for final concentration is 0.5 µM but it can be optimized in a range of 0.2–1.0 µM, if need

Table 2. Cycling instructions.

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	94°C	10 min	94°C	10 s	1
Denaturation (see 5.1)	94°C	15 s – 1 min	94°C	15 s–1 min	25–35
Annealing (see 5.2)	–	–	T <sub>m</sub> -5°C	10–30 s	
Extension (see 5.3)	72°C	1 min/kb	72°C	1 min/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 enzyme depends on the amount of template and the length of the PCR product. Usually 1.2 units of DyNAzyme II Hot Start DNA Polymerase per 50 µl reaction gives good results, but optimal amounts can range between 0.75–2 units per 50 µl reaction. DyNAzyme II Hot Start DNA Polymerase amplifies DNA targets up to 3 kb, but optimal results are obtained with amplicons of ≤ 1 kb.

#### 4.2 Incorporation of nucleotide analogs

DyNAzyme II Hot Start DNA Polymerase incorporates 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 as a source of template, the volume of the template should not exceed 10 % of the final PCR reaction volume.

### 5. Notes about cycling conditions

#### 5.1 Denaturation

DyNAzyme II Hot Start DNA Polymerase is activated by an initial 10-minute denaturation step at 94°C. After the initial denaturation, keep the denaturation time as short as possible.

#### 5.2 Primer annealing

Primer annealing for 30 seconds or less at the highest temperature that will permit annealing of the primer to the template is recommended. Instructions for T<sub>m</sub> calculation and a link to a calculator using the nearest-neighbor method<sup>1</sup> can be found on Thermo Scientific website ([www.thermoscientific.com/pcrwebtools](http://www.thermoscientific.com/pcrwebtools)). Two-step cycling without annealing step is recommended for high T<sub>m</sub> primer pairs.

#### 5.3 Extension

Extension for one minute per kilobase at 72°C is recommended.

### 6. Troubleshooting

No product at all or low yield	
<ul style="list-style-type: none"> <li>• Repeat and make sure that there are no pipetting errors.</li> <li>• Optimize denaturation time. Remember to add the 10-minute initial denaturation.</li> <li>• Titrate template amount.</li> <li>• Template DNA may be damaged. Use carefully purified template.</li> </ul>	<ul style="list-style-type: none"> <li>• Use high quality dNTPs.</li> <li>• Increase extension time.</li> <li>• Increase the number of cycles.</li> <li>• Decrease annealing temperature.</li> <li>• Add DMSO (e.g. 5 %).</li> <li>• Check the purity and concentration of the primers.</li> <li>• Check primer design.</li> </ul>
Non-specific products - High molecular weight smears	
<ul style="list-style-type: none"> <li>• Decrease the enzyme concentration</li> <li>• Shorten extension time.</li> <li>• Reduce total number of cycles.</li> </ul>	<ul style="list-style-type: none"> <li>• Increase annealing temperature or try 2-step protocol</li> <li>• Decrease primer concentration.</li> </ul>
Non-specific products - Low molecular weight discrete bands	
<ul style="list-style-type: none"> <li>• Increase annealing temperature</li> <li>• Shorten extension time.</li> </ul>	<ul style="list-style-type: none"> <li>• Titrate template amount.</li> <li>• Decrease primer concentration.</li> <li>• Design new primers.</li> </ul>

### 7. Component specifications

#### 7.1 DyNAzyme II Hot Start DNA Polymerase (F-504)

DyNAzyme II Hot Start DNA Polymerase is a modified form of DyNAzyme II DNA Polymerase, which is purified from an *E. coli* strain expressing DyNAzyme DNA Polymerase from *Thermus brockianus*.

**Storage buffer:** 20 mM Tris-HCl (pH 9.0 at 25°C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, stabilizers 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. The enzyme is activated by heating for 20 min at 90°C prior to activity measurement.

**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-<sup>32</sup>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 [<sup>3</sup>H] ssDNA (2x10<sup>5</sup> 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.

### 7.2 DyNAzyme II Hot Start Reaction Buffer (F-522)

In final 1x reaction concentration the DyNAzyme II Hot Start Reaction Buffer contains 15 mM Tris-HCl (pH 8.2 at 25°C), 2.5 mM MgCl<sub>2</sub>, 30 mM KCl, 5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 0.02 % bovine serum albumin.

## 8. References

1. Breslauer K.J. *et al.* (1986) *PNAS* 83: 3746–3750.

### Shipping and storage

DyNAzyme II Hot Start 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)

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