

DyNAzyme™ II

PCR Master Mix

Product codes:

F-508S, 200 reactions

F-508L, 1000 reactions

Stable for six months from the assay date. Recommended storage temperature -20°C . After thawing the mix can be refrozen or optionally stored at $+4^{\circ}\text{C}$ for three months.

1. Introduction

Finnzymes' DyNAzyme™ II DNA Polymerase is a recombinant thermostable DNA polymerase purified from an *E.coli* strain expressing the cloned DyNAzyme DNA Polymerase gene from *Thermus brockianus*. DyNAzyme II DNA Polymerase possesses the following activities: 5'→3' DNA polymerase activity and 5'→3' exonuclease activity.

DyNAzyme II PCR Master Mix is a 2x master mix containing DyNAzyme II DNA Polymerase, nucleotides, and optimized reaction buffer including MgCl_2 . Only template and primers need to be added by the user.

Advantages

- Better thermal stability than that of *Taq* DNA polymerase
- Tolerates DMSO better than *Taq* DNA polymerase
- High purity, no contaminating activities
- Easy to use
- High batch-to-batch consistency

2. Package Information

F-508S	200 reactions in 50 μl volume 2x DyNAzyme™ II PCR Master Mix (4 x 1.25 ml) Contains: 0.04 U/ μl DyNAzyme™ II DNA Polymerase, 20 mM Tris-HCl (pH 8.8 at 25°C), 3 MgCl_2 , 100 mM KCl, stabilizers and 400 μM of each dNTP
F-508L	1000 reactions in 50 μl volume 2x DyNAzyme™ II PCR Master Mix (20 x 1.25 ml) Contains: 0.04 U/ μl DyNAzyme™ II DNA Polymerase, 20 mM Tris-HCl (pH 8.8 at 25°C), 3 MgCl_2 , 100 mM KCl, stabilizers and 400 μM of each dNTP

3. Setting up PCR Reactions Using DyNAzyme™ II PCR Master Mix

Carefully mix and centrifuge all tubes before opening to improve recovery. PCR reactions should be set up on ice.

Table 1. Pipetting instructions.

Component	Volume / 50 μl reaction	Volume / 20 μl reaction	Final conc.
H_2O	add to 50 μl	add to 20 μl	
2x DyNAzyme™ II PCR Master Mix	25 μl	10 μl	1x
primer A*	x μl	x μl	0.5 μM
primer B*	x μl	x μl	0.5 μM
template DNA	x μl	x μl	

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

4. Notes about Reaction Components

4.1 Enzyme

In DyNAzyme II PCR Master Mix the enzyme concentration is optimized. When pipetted according to the instructions, the final concentration is 1 U of enzyme in a 50 μl reaction.

4.2 Mg^{2+} concentration and dNTP concentration

The DyNAzyme II PCR Master Mix provides 1.5 mM MgCl_2 and 200 μM dNTP in final reaction concentration.

4.3 Template

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

4.4 PCR additives

DyNAzyme II DNA Polymerase tolerates the high DMSO concentrations (up to 10 %) needed for opening of difficult templates. Other additives which help DNA denaturation can also be used with DyNAzyme II DNA Polymerase (formamide, glycerol, betaine, and combinations of these). We recommend to use co-solvents in the following concentrations: DMSO 2-10 %, Formamide 2-10 %, Glycerol 5-10 %, or combinations of these. 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 $^{\circ}\text{C}$.¹

5. Cycling Conditions

Table 2. General cycling instructions.

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	94 $^{\circ}\text{C}$	1-2 min	94 $^{\circ}\text{C}$	1-2 min	1
Denaturation	94 $^{\circ}\text{C}$	15 s-1 min	94 $^{\circ}\text{C}$	15 s-1 min	25-35
Annealing (see 6.2)	–	–	$T_m - 5^{\circ}\text{C}$	10-30 s	
Extension	72 $^{\circ}\text{C}$	40 s/1 kb	72 $^{\circ}\text{C}$	40 s/1 kb	
Final extension	72 $^{\circ}\text{C}$ 4 $^{\circ}\text{C}$	5-10 min hold	72 $^{\circ}\text{C}$ 4 $^{\circ}\text{C}$	5-10 min hold	1

6. Notes about Cycling Conditions

6.1 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 cyclor.

6.2 Primer annealing

We suggest the primer to be annealed for one minute or less at the highest temperature that will permit annealing of the primer to the template. The primer T_m's should be calculated with the nearest-neighbor method² as results from primer T_m calculations can vary significantly depending on the method used. A guideline for DyNAzyme II DNA Polymerase is to use an annealing temperature 5°C below the lower T_m of the primers. Instructions for T_m calculation and a link to a calculator using the nearest-neighbor method can be found on Finnzymes website (www.finnzymes.com). Two-step cycling without an annealing step is recommended for high T_m primer pairs.

6.3 Extension

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

7. Troubleshooting

No product at all or low yield
<ul style="list-style-type: none">• Repeat and make sure that there are no pipetting errors.• Titrate template amount.• Template DNA may be damaged. Use carefully purified template.• Increase extension time.• Increase the number of cycles.• Decrease annealing temperature.• Titrate DMSO (2-8 %) in the reaction (see section 4.4).• Optimize the denaturation time.• Check the condition of the primers.• Check the purity and concentration of the primers.
Non-specific products - High molecular weight smears
<ul style="list-style-type: none">• Shorten extension time (see section 6.3).• Reduce total number of cycles.• Increase annealing temperature or try 2-step protocol (see section 6.2).• Vary denaturation temperature (see section 6.1).• Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands
<ul style="list-style-type: none">• Increase annealing temperature (see section 6.2).• Shorten extension time (see section 6.3).• Titrate template amount.• Decrease primer concentration.• Design new primers.

8. Component Specifications of DyNAzyme™ II PCR Master Mix

2x DyNAzyme II PCR Master Mix contains 0.04 U/μl DyNAzyme II DNA Polymerase, 20 mM Tris-HCl (pH 8.8 at 25°C), 3 MgCl₂, 100 mM KCl, stabilizers and 400 μM of each dNTP

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. **DNA amplification assay:** Performance in PCR is tested by the amplification of 500 bp λ DNA and 6 kb M13 DNA.

9. References

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

Storage and shipping

The DyNAzyme II PCR Master Mix is shipped on gel ice. Upon arrival, store the components at -20°C. The DyNAzyme II PCR Master Mix is stable for six months from the assay date when stored and handled properly. After thawing the DyNAzyme II PCR Master Mix can be refrozen or optionally stored at +4°C for three months.

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