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Package insert

S7 Fusion Polymerase™

V2-0
Product no. MD-S7-100 / MD-S7-500
Biozym Art. nr.: 332530S, 332530L

1. Introduction

S7 Fusion Polymerase™ is a high-fidelity DNA polymerase that offers extraordinary performance in PCR applications. The polymerase is fused to the processivity enhancing Sso7d domain from *Sulfolobus solfataricus*, making S7 Fusion Polymerase™ one of the fastest and most robust PCR polymerases on the market. The polymerase possesses a 3' -> 5' exonuclease activity and it generates blunt ends in PCR.

2. Reagent kit components

Component name	Product number	MD-S7-100	MD-S7-500
S7 Fusion Polymerase™ 2 U/µl	MD-E604S	100 U, 50 µl	-
S7 Fusion Polymerase™ 2 U/µl	MD-E604L	-	500 U, 250 µl
5X HF buffer	MD-B704	2 x 1,5 ml	6 x 1,5 ml
5X GC buffer	MD-B703	1,5 ml	2 x 1,5 ml
50 mM $MgCl_2$	MD-M501	1,5 ml	2 x 1,5 ml
DMSO	MD-D502	500 µl	500 µl

3. Shipping and storage

The S7 Fusion Polymerase™ reagents are packed into a plastic pouch and shipped on gel ice. After reception, the product should be stored at -20°C.

If stored under the recommended conditions the product will maintain its performance until the expiry date.

4. Guidelines for using S7 Fusion Polymerase™

PCR setup

Prepare the PCR reactions according to the table below. Add S7 Fusion Polymerase™ last.

	20 µl reaction	50 µl reaction	Final concentration
Water	Add to 20 µl	Add to 50 µl	
5X HF or 5X GC buffer	4 µl	10 µl	1X
10 mM dNTPs	0,4 µl	1 µl	200 µM each
Forward primer	X µl	X µl	0,5 µM
Reverse primer	X µl	X µl	0,5 µM
Template DNA	See instruction for details		
S7 Fusion Polymerase™	0,2 µl	0,5 µl	0,02 U/µl

Buffers and additives

Two buffers are provided with the S7 Fusion Polymerase™. The HF buffer is recommended for high-fidelity applications, as the error rate in PCR is lower than with the GC buffer. The GC buffer is recommended for GC-rich templates or long PCR reactions. The GC buffer may contain some precipitate after thawing; the precipitate dissolves upon heating.

GC-rich or long PCR reactions might need DMSO in order to produce a satisfactory yield. Add 3% DMSO (e.g. 1,5 µl per 50 µl reaction) as a starting point for DMSO optimization. High concentrations of DMSO affect the Tm of the primers. As a general guideline, a final concentration of 10% DMSO requires a 5°C decrease in the annealing temperature. Other PCR additives such as formamide, glycerol and betaine can also be used for difficult templates.

$MgCl_2$

The 5X HF and 5X GC buffers contain an optimal concentration of $MgCl_2$. Higher Mg^{2+} concentrations can increase PCR yield but decrease specificity, whereas low Mg^{2+} concentrations may lead to low or no yield. An increase of the $MgCl_2$ concentration may be needed if the template or primers contain Mg^{2+} binding chelators, such as EDTA or EGTA. Also, if there is an excess of dNTPs present in the reaction (e.g. when using cDNA synthesis mix as template), the amount of $MgCl_2$ should be increased. When adjusting the $MgCl_2$ concentration, make the increments in 0,2 mM steps (e.g. 0,2 µl 50 mM $MgCl_2$ per 50 µl reaction).

dNTPs
It is recommended to use 200 µM of each dNTP. NOTE: Do not use dUTP, since S7 Fusion Polymerase™ cannot amplify templates containing dUTP.

Template DNA
The amount of needed template DNA is dependent on the complexity. For low complexity templates, such as plasmid or lambda DNA, a few picograms to a few nanograms of template is sufficient in a 50 µl PCR reaction. For high complexity genomic templates, 50-250 ng per 50 µl PCR reaction can be used. If using a cDNA synthesis reaction mixture as template, increasing the template amount to more than 10% of the PCR mixture does generally not increase yield.

Polymerase
It is recommended to use 1 unit S7 Fusion Polymerase™ per 50 µl PCR reaction, but the optimum can vary between 0,5 and 2 units per 50 µl PCR reaction. For amplicons that are several kb long, no more than 2 units per 50 µl should be used.

PCR cycling
For optimal performance, the following PCR cycling protocols are recommended:

2-step protocol

Cycles	Step	Temperature	Time
1	Initial denaturation	98°C	30 s
25-35	Denaturation	98°C	5-10 s
	Annealing and extension	72°C	15-30 s/kb
1	Final extension	72°C	5-10 min
1	Cooling	4°C	hold

3-step protocol

Cycles	Step	Temperature	Time
1	Initial denaturation	98°C	30 s
25-35	Denaturation	98°C	5-10 s
	Annealing	X°C	10-30 s
	Extension	72°C	15-30 s/kb
1	Final extension	72°C	5-10 min
1	Cooling	4°C	hold

Denaturation
A short denaturation time of 5-10 seconds at 98°C is sufficient for most templates.

Annealing
The S7 Fusion Polymerase™ requires higher annealing temperatures than most other PCR polymerases. The higher annealing temperature allows for more frequent use of a two-step protocol, where the annealing and extension steps are combined. As a rule of thumb, for primers >20 nt in length, the annealing temperature should be the Tm of the lower Tm primer +3°C. For primers ≤20 nt, the annealing temperature should be the Tm of the lower Tm primer. The two-step protocol should be used when the annealing temperatures of the primers are 72°C or higher.

Extension
Amplicon complexity and length determine the required extension time. Simple templates require 15 s/kb extension time, whereas more complex templates need 30 s/kb.

5. Specifications

Storage buffer
20 mM Tris-HCl pH 8,0, 100 mM KCl, 0,1 mM EDTA, 0,2 mg/ml BSA, stabilizers and 50% glycerol.

Unit definition
One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTPs into a polynucleotide fraction at 72°C in 30 min.

Endonuclease contamination assay
No conversion to nicked form is detected after incubating S7 Fusion Polymerase™ with supercoiled ΦX174 DNA.

Exonuclease contamination assay
No contaminating exonuclease activity is detected after incubating S7 Fusion Polymerase™ with radiolabeled DNA.

DNA amplification
S7 Fusion Polymerase™ amplifies a 7,5 kb genomic DNA fragment and a 10 kb lambda DNA fragment.

Safety
This product should be handled only by persons trained in laboratory techniques.

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