

Instruction manual KOD EXO(-) 2605

KOD exo(-) DNA Polymerase

KODND-109B 1 KU

Store at -20°C

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[1] Introduction

Description

KOD exo(-) is a 3'→5' exonuclease minus mutant developed based on KOD DNA polymerase from a hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1¹⁾²⁾. KOD exo(-) shows excellent elongation ability and PCR efficiency.

Features

-A fast mode cycle can be applied.

[2] Components

This reagent includes the following components;

KOD exo(-) (2.5 U/μL)
10× Buffer*

*The buffer contains 12mM MgCl₂.

[3] Protocol

1. Standard reaction setup

The following procedure is designed for use with the components provided in this kit. Before preparing the mixture, thaw all components completely except for the enzyme solution.

Component	Volume	Final Concentration
10x buffer for KOD exo(-)*	5 μL	1×
2mM dNTPs**	5 μL	0.2 mM each
10pmol /μL Primer #1	0.5~1 μL	0.1~0.2 μM
10pmol /μL Primer #2	0.5~1 μL	0.1~0.2 μM
Template DNA	X μL	{ Genomic DNA ~1000 ng / 50 μL Plasmid DNA ~50 ng / 50 μL cDNA ~200 ng (RNA equiv.) / 50 μL
PCR grade water	Y μL	
KOD exo(-) (2.5 U/μL)	0.5 μL	
Total reaction volume	50 μL	

*The buffer contains 12mM MgCl₂. For the fast mode cycle, an extra MgCl₂ should be added up to 7.2mM (final concentration).

** Do not use dNTPs from other kits or companies

Notes:

- For PCR reactions, thin-wall tubes are recommended. A total reaction volume of 50 μ L is also recommended.
- The addition of DMSO (final conc. 2-5%) might be effective for amplification of GC-rich targets. Decreased PCR fidelity has been confirmed to not take place with DMSO.
- Contaminated RNA (used for cDNA) or genomic DNA inhibits the PCR reaction by chelating Mg^{2+} . PCR should be performed using template DNA containing <200 ng RNA component.
- Primers should be 22~34 bases, with a melting temperature (T_m) > 60°C. PCR primers should be designed according to the general guidelines.

2. Cycling condition

The following cycling steps are recommended.

(1) Normal cycle (3-step cycle)

< 3-step cycle >	
Pre-denaturation :	94°C, 2min.
Denaturation :	94°C, 15sec.
Annealing :	(T_m ~ T_m -5) °C, 30 sec.
Extension :	72~74 °C, 30 sec. /kb

25~35 cycles

(2) Normal cycle (2-step cycle)

This cycle is suitable for the amplification >4kb targets.

< 2-step cycle >	
Pre-denaturation :	94 °C, 2min.
Denaturation :	94 °C, 15sec.
Annealing :	68~70 °C, 30 sec. /kb

25~35 cycles

(3) Fast mode cycle

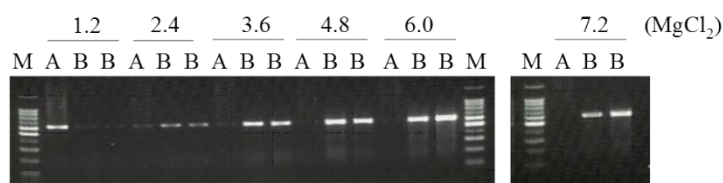
This cycle can be applied to <1.5kb targets. In this cycle, an extra $MgCl_2$ should be added up to 7.2 mM (final concentration).

< 3-step cycle >	
Denaturation :	95 °C, 0 sec.
Annealing :	(T_m ~ T_m -5) °C, 0 sec.
Extension :	72°C, 10~15 sec./kb

25~35 cycles

[4] Example

Example 1. Fast mode cycle (1)

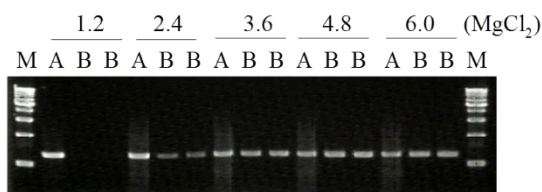


Reaction volume : 10 μ l
 Template: Human genomic DNA 5 ng
 Target: β -globin 582 bp
 KOD exo(-): 1.25 U
 dNTPs: 0.2 mM each (final)
 Primer: 0.2 μ M each (final)
 MgCl₂: 1.2 mM-7.2mM (final)

A: ABI 9700
 B: Speed cycler (ramp: Max)
 M: 100bp Ladder Marker

95 °C, 0 sec. ←
 60 °C, 0 sec. — 30 cycles
 72 °C, 5 sec. —

Example 2. Fast mode cycle (2)



Reaction volume : 10 μ l
 Template: Human genomic DNA 5 ng
 Target: β -globin 1.3 kb
 KOD exo(-): 1.25 U
 dNTPs: 0.2 mM each (final)
 Primer: 0.2 μ M each (final)
 MgCl₂: 1.2 mM-6.0mM (final)

A: ABI 9700
 B: Speed cycler (ramp: Max)
 M: 1kb Ladder Marker

95 °C, 0 sec. ←
 60 °C, 0 sec. — 30 cycles
 72 °C, 20 sec. —

[5] References

- 1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.*, 63: 4504-10 (1997)
- 2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.*, 306: 469-77 (2001)