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Instruction manual KOD EXO(-) 2004

# KOD exo(-) DNA Polymerase

250 U 200 reactions

**Store at -20°C**

Contents




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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* KOD1[1) 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*
2mM dNTPs

*The buffer contains 12mM $MgCl_2$.

## [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 l | 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<br>Plasmid DNA ~50 ng / 50 μL<br>cDNA ~200 ng（RNA equiv.) / 50 μL |
| PCR grade water | Y μL | |
| KOD exo(-) (2.5 U/μL ) | 0.5 μL | 1.25 U / 50 μL |
| Total reaction volume | 50 μL | |

*The buffer contains 12mM $MgCl_2$. For the fast mode cycle, an extra $MgCl_2$ should be added up to 7.2mM (final concentration).

** Do not use dNTPs from other kits or companies

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Notes:

-For PCR reactions, thin-wall tubes are recommended. A total reaction volume of 50 µL 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 (Tm) > 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 : | (Tm~Tm-5) °C, 30 sec. | 25~35 cycles |
| Extension : | 72~74 °C, 30 sec. /kb | ↲ |

(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 : | (Tm~Tm-5) °C, 0 sec. | 25~35 cycles |
| Extension : | 72°C, 10~15 sec./kb | ↲ |

## [4] Example

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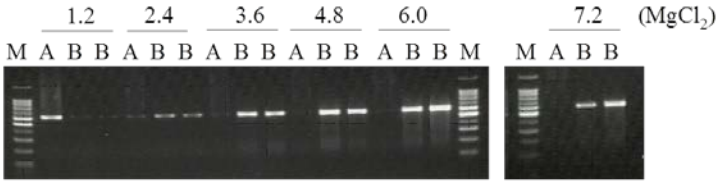
Example 1. Fast mode cycle (1)

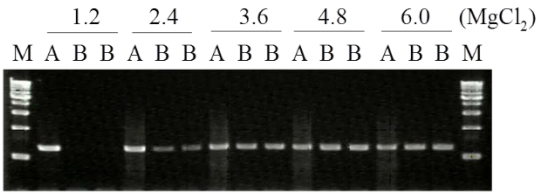



Reaction volume : 10 µl
Template: Human genomic DNA 5 ng
Target: ß-globin 582 bp
KOD exo(-): 1.25 U
dNTPs: 0.2 mM each (final)
Primer: 0.2 µM each (final)
$MgCl_2$: 1.2 mM~7.2mM (final)

95 ℃, 0 sec.
60 ℃, 0 sec.
72 ℃, 5 sec.
30 cycles

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

Example 2. Fast mode cycle (2)




Reaction volume : 10 µl
Template: Human genomic DNA 5 ng
Target: ß-globin 1.3 kb
KOD exo(-): 1.25 U
dNTPs: 0.2 mM each (final)
Primer: 0.2 µM each (final)
$MgCl_2$: 1.2 mM~6.0mM (final)

95 ℃, 0 sec.
60 ℃, 0 sec.
72 ℃, 20 sec.
30 cycles

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

## [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)

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