

# KOD -Plus-

KOD-201 200 U 200 reactions

Store at -20°C

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## CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.

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## [1] Introduction

### Description

KOD -Plus- is based on DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1<sup>1)2)</sup>. KOD -Plus- exhibits excellent high PCR fidelity and efficiency. The enzyme solution of KOD -Plus- contains two types of anti-KOD DNA polymerase antibodies that inhibit polymerase and 3'→5' exonuclease activity, thus allowing for Hot Start PCR<sup>3)</sup>. KOD -Plus- generates blunt-end PCR products, due to 3'→5' exonuclease (proof-reading) activity.

### Features

-Hot Start technology, using anti-KOD DNA polymerase antibodies, results in highly efficient amplification (see Example 1).

-KOD -Plus- enables the following amplifications (maximum): 21 kb from lambda phage DNA, 12 kb from human genomic DNA, and 7 kb from cDNA.

-KOD DNA polymerase has strong 3'→5' exonuclease (proof-reading) activity. The PCR error ratio of KOD -Plus- is approx. 80 times less than Taq DNA polymerase.

Table. 1 PCR fidelity comparison of each PCR enzyme.

	Colony number		Mutation frequency (%)
	Total	Mutant	
KOD -Plus-	10,610	10	0.09
High fidelity PCR enzyme (A company)	10,900	68	0.62
Pfu based DNA polymerase	6,520	76	1.17
Taq DNA polymerase	10,560	780	7.39

\*PCR fidelity was based on the mutation frequency of PCR products using a positive-selection base assay with the *rpsL* gene<sup>4)</sup>.

## [2] Components

This reagent includes the following components for 200 reactions:

KOD -Plus- (1.0 U/μl) *	200 μl × 1
10× Buffer for KOD -Plus-	1.0 ml × 1
25 mM MgSO <sub>4</sub>	1.0 ml × 1
2 mM dNTPs	1.0 ml × 1

\*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3'→5' exonuclease activity.

## [3] Quality Testing

Quality check can be performed by amplifying the β-globin gene (3.6 Kb) and p53 gene (4.0 Kb).

#### [4] Primer Design

-Primers should be 22-34 bases with a melting temperature ( $T_m$ ) over 60°C. For amplification of a long target, 25-34 bases with high  $T_m$  values ( $\geq 65^\circ\text{C}$ ) are recommended. PCR primers should be designed according to the general guidelines.

#### [5] Cloning of PCR products

-KOD-Plus- generates blunt-end PCR products, due to 3'→5' exonuclease (proof-reading) activity. Therefore, the product can be cloned according to a blunt-end cloning method.

-PCR products of KOD-Plus- should be purified prior to restriction enzyme treatments. The 3'→5' exonuclease activity of KOD DNA polymerase remains after the PCR cycles.

#### [6] Protocol

##### 1. Standard reaction setup

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

Component	Volume	Final Concentration
10x Buffer for KOD -Plus-	5 $\mu\text{l}$	1x
2mM dNTPs*	5 $\mu\text{l}$	0.2 mM each
25mM MgSO <sub>4</sub>	2 $\mu\text{l}$	1.0 mM
10pmol/ $\mu\text{l}$ Primer #1	1.5 $\mu\text{l}$	0.3 $\mu\text{M}$
10pmol/ $\mu\text{l}$ Primer #2	1.5 $\mu\text{l}$	0.3 $\mu\text{M}$
Template DNA	X $\mu\text{l}$	{ Genomic DNA 10-200 ng/50 $\mu\text{l}$ Plasmid DNA 1-50 ng/50 $\mu\text{l}$ cDNA $\leq 100$ ng (RNA equiv.)/50 $\mu\text{l}$
PCR grade water	Y $\mu\text{l}$	
KOD-Plus- (1.0 U/ $\mu\text{l}$ )	1 $\mu\text{l}$	1.0 U / 50 $\mu\text{l}$
Total reaction volume	50 $\mu\text{l}$	

\* Do not use dNTPs from other kits or companies.

##### Notes:

-For PCR reactions, thin-wall tubes are recommended. A total reaction volume of 50  $\mu\text{l}$  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<sup>2+</sup>. PCR should be performed using template DNA containing <100 ng RNA component.

## 2. Cycling conditions

The following cycling steps are recommended.

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### < 2-step cycle >

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Pre-denaturation:	94 °C, 2 min.	} 25-35 cycles
Denaturation:	94 °C, 15 sec.	
Extension:	68 °C, 1 min./kb	

**Note:** If the T<sub>m</sub> value of the primer is under 73 °C, the 3-step cycle is recommended.

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### < 3-step cycle >

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Pre-denaturation:	94 °C, 2 min.	} 25-35 cycles
Denaturation:	94 °C, 15 sec.	
Annealing:	T <sub>m</sub> -[5-10] °C*, 30 sec.	
Extension:	68 °C, 1 min./kb	

\*T<sub>m</sub> value of the primer minus 5°C-10°C

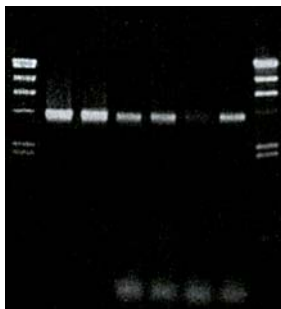
### Notes:

-Extension time should be set to 1min per 1 kb of target length.

[7] Examples

Example 1. Effect of Hot Start PCR on the generation of primer dimers.

M 1 2 3 4 5 6 M



Template: Human genomic DNA  
1,3,5: 50ng, 2,4,6: 100ng

Target: p53 gene 4kb

M:  $\lambda$  /HindIII Marker

1,2: KOD -Plus-

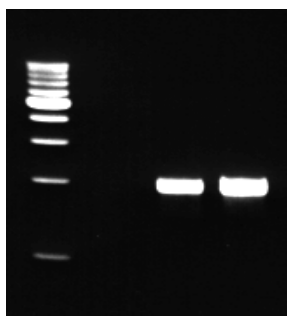
3,4: A company high fidelity enzyme

5,6: B company high fidelity enzyme

← Primer dimer

Example 2. Effect of addition of DMSO for GC-rich targets.

M 1 2 3



Template: Human genomic DNA

Target: TGF- $\beta$  gene (GC%=70) 2kb

M: 1kb Ladder Markers

1: KOD -Plus-, 0% DMSO

2: KOD -Plus-, 2% DMSO

3: KOD -Plus-, 5% DMSO

## [ 8 ] Troubleshooting

Symptom	Cause	Solution
No PCR product/low yield	Cycling conditions are not suitable.	Lower annealing temperature increments to a maximum of $T_m-10^{\circ}\text{C}$ . Increase the number of cycles by 2-5 cycles.
	Mg concentration is low	Increase the Mg concentration to 1.2-2 mM.
	High GC content of target sequence	Add DMSO 2-5%. <See Example 2>
	Primer is not good.	Check the quality of primers. Redesign primers.
	Quality and/or quantity of template DNA is not sufficient.	Check the quality of template DNA. RNA inhibits amplification. Increase the amount of template DNA.
Smearing/extra band	Cycling condition is not suitable.	Decrease the number of cycles by 2-5 cycles. Use step-down cycling.
	Primer is not good.	Check the quality of primers. Redesign primers.
	Too much template DNA	Reduce the amount of template DNA.
	Too much Mg	Reduce $\text{MgSO}_4$ to 0.8 mM.
	Too much enzyme	Reduce enzyme to 0.5-0.8 U/50 $\mu\text{l}$ .
Poor TA cloning efficiency	PCR products have blunt-ends.	Clone the PCR products according to general blunt-end cloning guidelines.

## [ 9 ] References

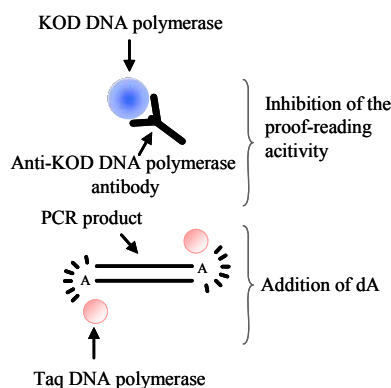
- 1) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T., *Appl Environ Microbiol.*, 63: 4504-10 (1997)
- 2) Hashimoto H, Nishioka M, Fujiwara S, Takagi M, Imanaka T, Inoue T and Kai Y, *J Mol Biol.*, 306: 469-77 (2001)
- 3) Mizuguchi H, Nakatsuji M, Fujiwara S, Takagi M and Imanaka T, *J Biochem.*, 126: 762-8 (1999)
- 4) Fujii S, Akiyama M, Aoki K, Sugaya Y, Higuchi K, Hiraoka M, Miki Y, Saitoh N, Yoshiyama K, Ihara K, Seki M, Ohtsubo E and Maki H, *J. Mol. Biol.*, 289: 835-850 (1999)

## [ 10 ] Related products

Product name	Package	Code No.
<b>TARget Clone -Plus-</b>	10 reactions	TAK-201
<b>10x A-attachment mix</b>	25 reactions	TAK-301
<b>Ligation high Ver.2</b>	750 $\mu$ l (100 reactions)	LGK-201

TARget Clone -Plus- is a high efficient TA cloning kit. The kit can be applied to the TA cloning of blunt-end PCR products amplified using KOD -Plus- [Code No. KOD-201] or KOD FX [Code No. KFX-101]. The kit contains pTA2 Vector, 2x Ligation Buffer, T4 DNA Ligase and 10x A-attachment Mix.

10 x A-attachment mix is a reagent comprising anti-KOD DNA polymerase antibody specific to KOD 3'→5' exonuclease activity (proof-reading activity), as well as Taq DNA polymerase, which exhibits terminal transferase activity. PCR products from KOD -Plus- [Code No. KOD-201] and KOD FX [Code No. KFX-101] possess blunt ends due to 3'→5' exonuclease activity of the KOD DNA polymerase. The 10 x A-attachment mix allows for PCR products to acquire overhanging dA at the 3'-ends. Products with 3'-dA overhangs can be directly cloned into arbitrary T-vectors using ligation reagents, such as Ligation high Ver.2 [Code No. LGK-201].



**Fig. Principle of the 10 x A-attachment mix**



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