KOD -Plus-

KOD-201 200 U 200 reactions

Store at -20°C

Contents

CAUTION
All reagents in this kit are intended for research purposes only. Not for diagnostic or clinical use. Please observe general laboratory safety precautions 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[1) 2)]. 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[3)]. 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 rate 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 [4)].

[2] Components

This kiy 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 $MgSO_4$	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 checks are performed by amplifying the β-globin gene (3.6 Kb) and p53 gene (4.0 Kb).

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[4] Primer Design

-Primers should be 22-34 bases with a melting temperature (Tm) over 60°C. For amplification of a long target, 25-34 bases with high Tm values (≥ 65°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 µl	1x
2mM dNTPs*	5 µl	0.2 mM each
25mM $MgSO_4$	2 µl	1.0 mM
10pmol/µl Primer #1	1.5 µl	0.3 µM
10pmol/µl Primer #2	1.5 µl	0.3 µM
Template DNA	X µl	Genomic DNA 10-200 ng/50 µl Plasmid DNA 1-50 ng/50 µl cDNA ≤ 100 ng (RNA equiv.)/50 µl
PCR grade water	Y µl	
KOD-Plus- (1.0 U/µl)	1 µl	1.0 U / 50 µl
Total reaction volume	50 µl	

* Do not use dNTPs from other kits or companies.

Notes:

-For PCR reactions, thin-wall tubes are recommended. A total reaction volume of 50 µl is also recommended.

-The addition of dimethyl sulfoxide (DMSO; final conc. 2-5%) might be effective for amplification of GC-rich targets. No decrease in PCR fidelity has been observed using DMSO.

-Contaminating RNA inhibits the PCR reaction by chelating Mg^{2+}. PCR should be performed using template DNA containing <100 ng of RNA.

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2. Cycling conditions

The following cycling steps are recommended.

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

Note: If the Tm value of the primer is under 73 °C, the 3-step cycle is recommended.

< 3-step cycle >		
Pre-denaturation:	94 °C, 2 min.	
Denaturation:	94 °C, 15 sec.	25-35 cycles
Annealing:	Tm-[5-10] °C*, 30 sec.	
Extension:	68 °C, 1 min./kb	

*Tm value of the primer minus 5°C-10°C

Notes:

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

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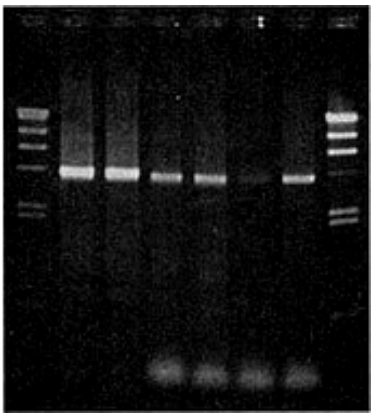
[7] Examples

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

The P53 gene (4 kb) was amplified by KOD-Plus-Ver.2 and high fidelity PCR enzymes from other companies using human genomic DNA as the template. KOD -Plus- successfully amplified the target genes without generating primer-dimmers.

M 1 2 3 4 5 6 M



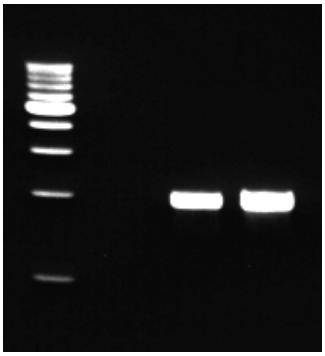
Template: Human genomic DNA
1,3,5: 50ng, 2,4,6: 100ng
Target: p53 gene 4kb
M: l/*Hin*d III Marker
1,2: KOD -Plus-
3,4: A company high fidelity enzyme
5,6: B company high fidelity enzyme

← Primer dimer

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

The TGF-b gene (2kb, GC%=70) was amplified using KOD-Plus-Ver.2 with or without DMSO. The addition of 2-3 % DMSO permitted effective amplification of the target.

M 1 2 3



Template: Human genomic DNA
Target: TGF-β gene (GC%=70) 2kb
M: 1kb Ladder Markers
1: KOD -Plus-, 0% DMSO
2: KOD -Plus-, 2% DMSO
3: KOD -Plus-, 5% DMSO

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[8] Troubleshooting

Symptom	Cause	Solution
No PCR product/low yield	Cycling conditions are not suitable.	Lower annealing temperature increments to a maximum of Tm-10°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 $MgSO_4$ to 0.8 mM.
	Too much enzyme	Reduce enzyme to 0.5-0.8 U/50 µl.
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. Bio*l., 289: 835-850 (1999)

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[10] Related products

Product name	Package	Code No.
TArget Clone™ -Plus-	10 reactions	TAK-201
10x A-attachment mix	25 reactions	TAK-301
Ligation high Ver.2	750 µl (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], KOD -Plus- Neo [Code No. KOD-401] 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].

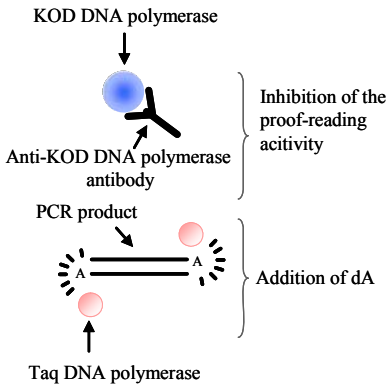

Fig. Principle of the 10 x A-attachment mix

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