Instruction manual KOD Dash 2109 F0939K

KOD Dash

LDP-101 250 U 200 reactions

Store at -20°C

Contents

CAUSION

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.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
bio_overseas@toyobo.jp

CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

[1] Introduction

Description

KOD Dash is a highly efficient DNA polymerase mixture developed based on the Barns' method[1]. This method uses a DNA polymerase which lacked a 3'→5' exonuclease (proofreading) activity and a small amount of an archaeal DNA polymerase with proofreading activity. In the reagent, the 3'→5' exonuclease activity-deficient mutant <KOD EXO(-)> of KOD DNA polymerase[2] and KOD DNA polymerase are used. Because the proofreading activity repairs misincorporated nucleotide bases causing the termination of a polymerase reaction, PCR with this mixed enzyme solution enables highly efficient amplification.

KOD Dash generates dA overhang-ended PCR products. Therefore, the PCR products can be cloned using a standard TA cloning method.

Features

-This enzyme mixture is effective for the amplification of various targets from a small template amount. The elongation ability of this enzyme mixture is much greater than that of the normal Taq DNA polymerase.

-This enzyme mixture shows greater elongation velocity than Taq DNA polymerase (2 folds) and Pfu DNA polymerase (6 folds) due to the property of KOD DNA polymerase.

-The PCR error ratio of this enzyme mixture is approximately 3 to 4 times less than that of Taq DNA polymerase.

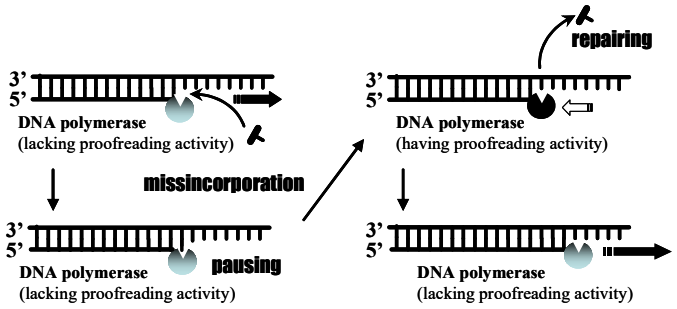


Fig. 1. The principles of the Barns' technology.

[2] Components

The provided reagents include the following components for 200 reactions:

KOD Dash (2.5 U/µl)	100 µl
10x PCR Buffer for KOD Dash*	1.2 ml
2 mM dNTPs	1.0 ml

*The final Mg concentration in the reaction mixture is 1.2 mM.

[3] Quality Testing

A quality check was performed by amplifying the human β-globin gene (8.5 kb) and the lambda phase gene (12 kb).

[4] Primer Design

PCR primers should be designed according to general guidelines. For the amplification of a long target (≥ 4 kb), the melting temperature (Tm) of the primers should be set over 72 °C.

[5] Cloning of PCR products

The PCR products obtained with amplification by KOD Dash can be cloned according to a standard TA-cloning method.

[6] Protocol

1. Standard reaction setup

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

*10x Buffer may precipitate. If precipitation occurs, dissolve it completely by overturning and mixing before use. The precipitate on the bottom of the tube can be easily dissolved by tapping it and then mixing it with the solution. If the precipitate is difficult to dissolve, repeat both heating the reagent at 50°C for 5 min and mixing it. Before preparing the reaction solution, mix each reagent thoroughly before use.

Component	Volume	Final Concentration
10x Buffer for KOD Dash	5 µl	1x
2 mM dNTPs*	5 µl	0.2 mM each
10 pmol/µl Primer #1	1 µl	0.2 µM
10 pmol/µl Primer #2	1 µl	0.2 µM
Template DNA	X µl	Genomic DNA 10-1000 ng/50 µl Plasmid DNA 1-50 ng/50 µl cDNA ≤1 µg（RNA equiv.)/50 µl *E. coli* cells (small amount)
PCR grade water	Y µl	
KOD Dash (2.5 U/µl)	0.5 µl	1.25 U / 50 µl
Total reaction volume	50 µl	

* Do not use dNTPs from other kits or companies.

Notes:

-Thin-wall tubes are recommended for PCR use. A total reaction volume of 50 µl is recommended.

-For amplification of a long target (≥ 8 kb), the use of a final concentration of 0.35 mM dNTPs may reduce non-specific amplification.

2. Cycling conditions

The following cycling steps are recommended.

(1) Cycling conditions for < 4kb targets.

< 3-step cycle >		
Pre-denaturation	94 °C, 0-2 min	
Denaturation	94 °C, 30 sec.	25-35 cycles
Annealing	Tm-[0-5] °C*, 1-5 sec.	
Extension	74 °C, 30 sec./kb	

*Tm value of the primer minus 0°C-5°C

(2) Cycling conditions for ≥ 4kb products.

< 2-step cycle >		
Pre-denaturation	94 °C, 0-2 min	
Denaturation	94 °C, 30 sec.	25-35 cycles
Extension	68-70 °C, 30 sec./kb	

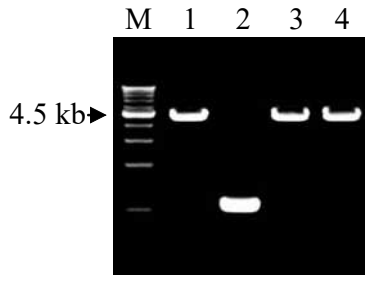
Note : If the Tm value of the primer is under 70 °C, the 3-step cycle is recommended.

Notes:

-Extension time should be set at 30 sec. per 1 kb of target length.
-For colony-direct PCR, the pre-denaturation step should be set for 4 min.

[7] Examples

Example 1. Colony-direct PCR using *E. coli* cells.



Lane 1: Insert (+)
Lane 2: Insert (-)
Lane 3: Insert (+)
Lane 4: Insert (+)

Template:Small amount of *E. coli* cells (JM109) from colonies
Insert: Transferrin receptor 4.5 kb
Vector: pBluescript II

PCR cycling condition:

94 ℃ 4 min.
98 ℃ 30 sec.
60 ℃ 5 sec.
72 ℃ 2.5 min. } 30 cycles

F primer: TCGAGGTCGACGGTATCGAT (20mer GC=55%)
R primer: CGCTCTAGAACTAGTGGATC (20mer GC=50%)

*Primers are designed on the vector.

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[8] Troubleshooting

Symptoms	Cause	Solutions
No PCR product / low yield	Cycling conditions are not optimal	Lower annealing temperature increments to a maximum of Tm-10 °C.
		Increase the number of cycles by 2-5 cycles.
	Primers are not good	Check the design and/or quality of primers.
	Too much *E. coli* cells (Colony direct PCR)	Decrease the amount of *E. coli* cells from colonies.
Smearing / extra band(s)	Cycling conditions are not optimal	Decrease the number of cycles by 2-5 cycles.

[9] Related products

Product name	Package	Code No.
Highly efficient cDNA synthesis kit **ReverTra Ace® -α-**	100 rxns	FSK-101
Highly efficient reverse transcriptase **ReverTra Ace®**	10,000 U	TRT-101
RNase inhibitor (Recombinant type)	2,500 U	SIN-201
Highly efficient Taq DNA polymerase **Blend Taq®**	250 U	BTQ-101
Highly efficient Taq DNA polymerase (Hot start version) **Blend Taq® -Plus-**	250 U	BTQ-201
High fidelity PCR enzyme **KOD -Plus-**	200 U	KOD-201
Highly reliable PCR enzyme **KOD FX**	200 U	KFX-101

[10] References

1) Barns WM, *Proc. Natl. Acad. Sci.* USA, 91: 2216-2220 (1994)

2) Takagi M, Nishioka M, Kakihara H, Kitabayashi M, Inoue H, Kawakami B, Oka M, and Imanaka T, *Appl Environ Microbiol*., 63: 4504-10 (1997)

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