# KOD -Plus-

KOD-201 200 U 200 reactions

**Store at -20°C**

## Contents



**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.

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

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

## [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 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 [4)].

## [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 $MgSO_4$ | 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 check can be 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<br>Plasmid DNA 1-50 ng/50 µl<br>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 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 <100 ng RNA component.

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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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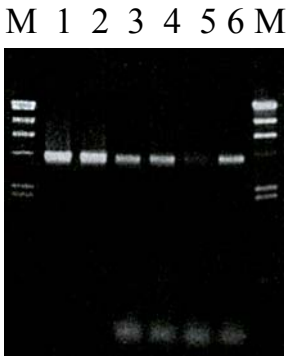
## [7] Examples

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



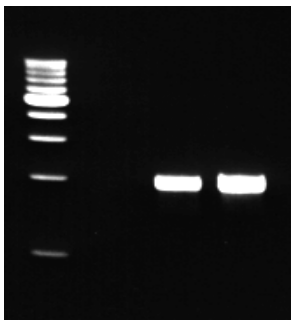

Template: Human genomic DNA
1,3,5: 50ng, 2,4,6: 100ng

Target: p53 gene 4kb

M: λ/*Hin*dⅢ 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.




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. |
|---|---|---|
| **10x A-attachment mix** | 25 reactions | TAK-301 |
| **Ligation high Ver.2** | 750 µl<br>(100 reactions) | LGK-201 |

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].

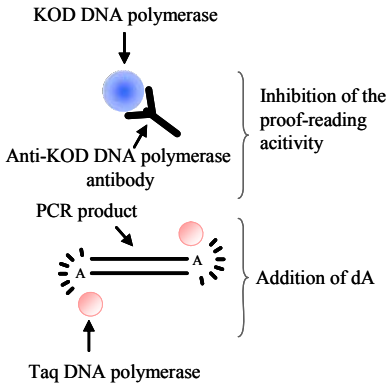


**Fig. Principle of the 10 x A-attachment mix**

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