Description

KOD DNA polymerase from Thermococcus kodakaraensis KOD is one of the most efficient thermostable PCR enzymes exhibiting higher accuracy and elongation velocity than any other commercially available DNA polymerase.The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5' →3' direction. The KOD DNA Polymerase also exhibits 3' →5'exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5' →3' exonuclease activity..

Primer Design

-Primers should be 22-34 bases with a melting temperature (Tm) over 60°C. Foramplification 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.

Cloning of PCR products

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

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

Protocol

1. Standard reaction setup

The following procedure is designed for use with the components provided in this kit.

Component	Volume	Final Concentration
PCR grade water	Y µl	
10×KOD Buffer with Mg^{2+}	5 µl	1x
2.5mM dNTPs*	4 µl	0.2 mM each
10pmol/µl Primer #1	1.5 µl	0.3 µM
10pmol/µl Primer #2	1.5 µl	0.3 µM Genomic DNA 10-200 ng/50 µl
Template DNA	X µl	Plasmid DNA 1-50 ng/50 µl cDNA ≤ 100 ng （RNA equiv.)/50 µl
KOD-Plus- (1.0 U/µl)	1 µl	1.0 U / 50 µl
Total reaction volume	50 µl	

- 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.

2. Cycling conditions

The following cycling steps are recommended.

< 2-step cycle >

Pre-denaturation: 94 °C , 2 min.
Denaturation: 94 °C, 15 sec.
Extension: 68 °C, 10 s～25 s./kb.
25-35 cycles

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.
Annealing: Tm-[5-10] °C, 30 sec.
Extension: 68 °C, 10 s～25 s./kb
25-35 cycles

Note: Tm value of the primer minus 5°C-10°C

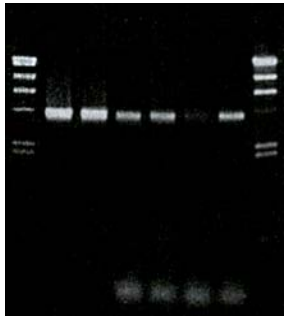
Notes:-Extension time should be set to , 10 s～25 s./kb of target length.

Step	Target size			
	< 500 bp	**500–1000 bp**	**1000–3000 bp**	**> 3000 bp**
1. Polymerase activation	95°C for 2 min	95°C for 2 min	95°C for 2 min	95°C for 2 min
2. Denature	95°C for 20 s	95°C for 20 s	95°C for 20 s	95°C for 20 s
3. Annealing	Lowest Primer Tm°C for 10 s			
4. Extension	70°C for 10 s/kb	70°C for 15 s/kb	70°C for 20 s/kb	70°C for 25 s/kb
Repeat steps 2–4	20–40 cycles. For more information see "Cycle number" below			

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: λ/*Hind*Ⅲ Marker

1,2: KOD

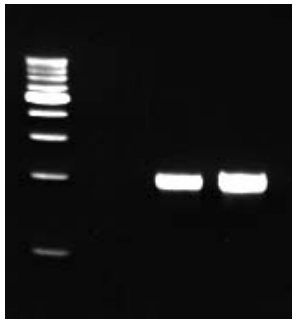
3,4: A company high fidelity enzyme

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-β gene (GC%=70) 2kb

M: 1kb Ladder Markers

1: KOD, 0% DMSO

2: KOD , 2% DMSO

3: KOD , 5% DMSO

KOD DNA Polymerase

包装内容	Cat: PC300	Cat: PC301	Cat: PC302
KOD -Plus- (1.25 U/μl)	250U	250U X4	250U X12
5×KOD Buffer Ⅰ with Mg^{2+}	1.0 ml × 1	1ml X12	1ml X12
5×KOD Buffer Ⅱ with Mg^{2+}	1.0 ml × 1	1ml X12	1ml X12

* 5×KOD Buffer Ⅱ含有 PCR 增强剂，可以同时做 PCR，择优使用；又可分为含 Mg2+和不含 Mg2+两种，用户可自选。不特别要求通常提供含 Mg2+的。

贮存条件：

-20℃保存

产品特性：

-----扩增准确性是 Taq 酶的约 83 倍。

---- 扩增速度是 Taq 酶的约 2 倍，pfu 酶的约 5 倍。

-----耐热性比 Taq 酶更好，在 100℃、1h 的热处理后仍有约 70%的活性，有利于高 GC 含量样品扩增。

-----产物为平末端