

Description

KOD FX is based on DNA polymerase from the hyperthermophilic Archaeon *Thermococcus kodakaraensis* KOD1. KOD FX results in much greater PCR success based on efficiency and elongation capabilities than KOD -Plus- or other Taq-based PCR enzymes. KOD FX enzyme solution contains two types of anti-KOD DNA polymerase antibodies that inhibit polymerase and 3'-5' exonuclease activities, thus allowing for Hot Start PCR. KOD FX generates blunt-end PCR products, due to 3'-5' exonuclease (proof-reading) activity.

Features

- This enzyme is effective for the amplification of GC-rich targets and crude samples.
- Hotstart technology using anti-KOD DNA polymerase antibodies enables highly efficient amplification.
- KOD FX enables the following amplifications (Maximum): 40kb from lambda phage DNA, 24kb from human genomic DNA, and 13.5kb from cDNA.
- The PCR error ratio is about 10 times less than that of Taq DNA polymerase

Components

This reagent includes the following components :

Cotents	GSX101-20T	GSX101-200T
KOD FX (1.0 U/ μ l) *	20ulx 1	200ulx 1
2x PCR Buffer **	500ulx 1	1.7 ml x 3
2 mM dNTPs	200ulx 1	1 ml x 2

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

** 2x PCR Buffer for KOD FX is a liquid (not congealed) when in storage at -20°C. Although it does congeal below -20°C, the quality is not affected.

Quality Testing

Quality check is performed by amplification of the tPA gene.

Primer Design

- Primers should be 22-35 bases with a melting temperature (T_m) over 60°C. For amplification of a long target, 25-35 bases with high T_m values ($\geq 65^\circ\text{C}$) are recommended. The primer T_m value for the 2-step or step-down cycles should be $\geq 65^\circ\text{C}$.
- For amplification of longer targets ($\geq 10\text{kb}$), primer Oligos should be cartridge- or HPLC- purified. Primers purified by gel filtration tend to result in smeared PCR products.

Cloning of PCR products

- KOD FX 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 FX 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 protocol 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.

Compents	Volume	Final Concentration
2x PCR buffer	25ul	1x
2mM dNTPs	10ul	0.4 mM each
10pmol /ul Primer F	1.5ul	0.3 uM
10pmol /ul Primer R	1.5ul	0.3 uM
KOD FX (1.0U/ul)	1ul	1.0 U / 50ul
Template DNA	Xul	Genomic DNA ≤ 200 ng Plasmid DNA ≤ 50 ng cDNA ≤ 200 ng(RNA equiv.) Crude sample $\leq 1-2$ ul
PCR grade water	up to 50ul	
Total	50ul	

* Do not use dNTPs from other kits or companies.

Notes:

- For PCR reaction, thin-wall tubes are recommended. Reaction setup to a total reaction volume of 50 ul is also recommended.
- Addition of DMSO (final conc. 2-5%) might be effective for amplification of GC-rich target. No decreasing of the PCR fidelity by adding DMSO has been confirmed.
- Crude samples (e.g., cultured animal cell suspension) should be added up to 2 ul for each 50 ul reaction.

2. Cycling conditions

The following cycling steps are recommended.

[2-Step Cycle]

Pre-denaturation :	94°C, 2 min.		20-40cycles
Denaturation :	98°C, 10 sec.		
Extension :	68°C, 1 min. /kb		

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

1

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[3-Step Cycle]

Pre-denaturation : 94°C, 2 min.
 Denaturation : 98°C, 10 sec.
 Annealing : T_m-[5-10]°C*, 30 sec.
 Extension : 68°C, 1 min. /kb

20-40 cycles

- T_m value of the primer minus 5°C-10°C

[Step Down Cycle]

Pre-denaturation : 94°C, 2 min.
 Denaturation : 98°C, 10 sec. ← **5cycles**
 Extension : 74°C, 1 min. /kb
 Denaturation : 98°C, 10 sec. ← **5cycles**
 Extension : 72°C, 1 min. /kb
 Denaturation : 98°C, 10 sec. ← **5cycles**
 Extension : 70°C, 1 min. /kb
 Denaturation : 98°C, 10 sec. ← **15-25 cycles**
 Extension : 68°C, 1 min. /kb
 Extension : 68°C, 7 min.

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

Notes:

- Extension time should be set at 1min. per 1kbp of target length. Although even 30 sec./ kb will give amplification in many cases, amplification efficiency or reliability may be decreased.
- Because this enzyme possesses an extremely high amplification efficiency, 25-30 cycles usually give sufficient amplification. For a low-copy number target, or over 10 kb target length, 30-40 cycles are recommended.
- The step-down cycle condition is effective for trouble shooting a smear amplification in a long-distance PCR (>10kb).

Trouble shooting

Symptom	Cause	Solution
No PCR product / Low yield	Cycling condition is not suitable.	● Use 3-step instead of 2-step cycling. Lower annealing temperature in 3°C decrements up to T _m -10°C.
		● Increase the number of cycles by 2-5 cycles.
	Primer is not good.	● Check the quality of primers.
		● Redesign primers.
	Template DNA is not good in quality and/or quantity.	● Check the quality of template DNA.
		● Increase the amount of template DNA

Symptom	Cause	Solution
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 enzyme	● Reduce Enzyme to 0.5-0.8U/ 50 μ l	

Citation and References

- 1) Nature Genetics(06 Apr 2009), 358, Letters.
- 2) Mol. Cancer Res.,Dec 2008; 6: 1937 - 1945.
- 3) PNAS, Feb 2009; 106: 2683 - 2687.
- 4) Hypertension Research32, 133 - 139 (23 Jan 2009).
- 5) Appl Environ Microbiol., 63: 4504-10 (1997)
- 6) Mol Biol., 306: 469-77 (2001)
- 7) J Biochem., 126:762-8 (1999)
- 8) J Biochem. 125(6): 983-6 (1999).
- 9) J Mol Biol. 306(3): 469-77 (2001).
- 10) J Biochem. 131(2): 183-91 (2002).
- 11) Int J Mol Med. 9(2): 107-12 (2002).

Example

- **Amplification of a GC-rich target**
90%GC rich target can be amplified
- **Amplification of long targets**
40kb from lambda phage DNA, 24kb from human genomic DNA, and 13.5kb from cDNA
- **Amplification of small amount of template**
1ng template DNA can be amplified
- **Amplification of crude samples**
 1. Whole Blood
 2. Cultured cells (Jurkat cells).
 3. Crude mouse tail lysate
 4. Crude Plant (leaf or seed) lysate
 5. Colony PCR (Yeast or E. Coli)
 6. Crude hair sample

More detail information on another page

LIMITED LICENSE and MSDS

- If you need it, please contact us.

CAUTION

- All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes.
- Please store this product at -20°C. Spin before using it.

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