

# Fidelity and reliability in PCR using KOD Hot Start

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*The performance of KOD Hot Start DNA Polymerase is compared with PfuUltra and PfuTurbo enzymes. The KOD enzyme exhibits higher yield with a lower mutation frequency.*

The family of recombinant *Thermococcus kodakaraensis* KOD1 DNA polymerases offers the highest elongation rates and processivity in DNA amplification, and possesses one of the highest fidelities among the thermostable DNA polymerases used for PCR. This article demonstrates the superior DNA yield in PCR amplification with KOD Hot Start DNA Polymerase\* as compared to the performance of two DNA polymerases isolated from the extremophile *Pyrococcus furiosus*. In addition, KOD Hot Start DNA polymerase shows excellent fidelity compared to *Taq*, *PfuUltra*<sup>™</sup>, and *PfuTurbo*<sup>®</sup> DNA polymerases (Stratagene).

KOD Hot Start DNA Polymerase is a premixed complex of KOD HiFi DNA Polymerase, a recombinant form of *T. kodakaraensis* KOD1 DNA polymerase (1, 2), and two monoclonal antibodies that inhibit DNA polymerase and 3'–5' exonuclease activities at ambient temperatures (3).

## Advantages of KOD Hot Start DNA Polymerase

KOD Hot Start DNA Polymerase combines the high fidelity, rapid extension speed, and outstanding processivity of KOD HiFi DNA Polymerase (Table 1) with the high specificity that results from antibody-mediated hot start technology.

Because most of the mispriming events that can occur throughout setup at ambient temperatures and during the initial increase in temperature are avoided, non-specific amplification is reduced. Similarly, primer degradation due to exonuclease activity at the ambient setup temperature is effectively inhibited. These features offer a distinct advantage for

robotic PCR applications in which preparations may be held at ambient temperatures for varying periods before the cycling process begins.

The 3'–5' exonuclease-dependent proofreading activity of this DNA poly-

**Table 1. DNA polymerase comparison: KOD HiFi, Pfu, and Taq**

Enzyme	KOD HiFi DNA Polymerase	<i>Pfu</i> DNA Polymerase	<i>Taq</i> DNA Polymerase
Species	<i>Thermococcus kodakaraensis</i>	<i>Pyrococcus furiosus</i>	<i>Thermus aquaticus</i> YT-1
Fidelity <sup>†</sup> (mutation frequency)	0.0035	0.0039	0.013
Elongation rate (bases/second)	106–138	25	61
Processivity (nucleotide bases)	> 300	< 20	not determined

<sup>†</sup> Fidelity was measured as mutation frequency in PCR products using a sensitive blue/white phenotypic assay with a 5.2 kbp *lacZ* plasmid as template (2).

merase results in a lower PCR mutation frequency than any other commercially available DNA polymerase. Elongation rates and processivities that are, respectively, 5 times and 10 to 15 times higher than *Pfu* DNA polymerase, result in a robust yield of a highly accurate product in a short reaction time.

KOD Hot Start amplifies problematic GC-rich sequences, genomic DNA templates up to 12 kbp long, and plasmid and

$\lambda$  DNA templates up to 21 kbp long. It produces blunt-ended PCR fragments suitable for cloning with Novagen's Perfectly Blunt<sup>®</sup> Cloning Kits or other blunt-end cloning methods.

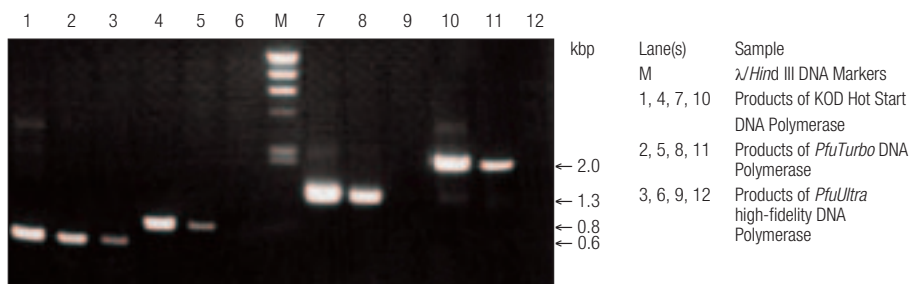
## PCR Yield

We compared the DNA yield in PCR reactions performed with three DNA polymerases: KOD Hot Start, *PfuTurbo*, and *PfuUltra* high-fidelity. PCR reactions using 100 ng human genomic DNA templates were performed according to the following protocol: initial denaturation at 95°C for 2 minutes, 35 amplification cycles (30 seconds at 95°C, 30 seconds at 60°C, 2 minutes at 72°C), and final extension at 72°C for 2 minutes. (Note: 72°C is an optimal extension temperature for both *Pfu* enzymes but is sub-optimal for KOD Hot Start genomic DNA amplification.) The primers were designed to amplify DNA fragments of 0.6, 0.8, 1.3, and 2.0 kbp. As shown in Figure 1, the

reaction performed with KOD Hot Start DNA Polymerase resulted in the highest yield of all four target PCR fragments. Both *PfuTurbo* and *PfuUltra* high-fidelity DNA polymerases generated lower yields of products under the same conditions.

## PCR Fidelity

The fidelity of replication was measured as the mutation frequency in PCR products using a modified *rpsL*<sup>+</sup> fidelity



**Figure 1. Performance of KOD Hot Start, PfuTurbo, and PfuUltra high-fidelity DNA polymerases in amplification of human genomic DNA**

The indicated DNA fragments were amplified using sets of specific primers and 100 ng human genomic DNA. Reactions contained 0.3  $\mu$ M each primer, the appropriate PCR buffer, 0.2 mM each dNTP, and 1 mM  $MgSO_4$  (KOD Hot Start reactions) or approximately 2 mM  $MgCl_2$  (*PfuTurbo* and *PfuUltra* reactions, included in 1X PCR buffer). Cycling parameters are described in the text. Samples (equal volumes) were analyzed by agarose gel electrophoresis (1.2% TAE) and stained with ethidium bromide.

\* Manufactured by Toyobo and distributed by EMD Biosciences Inc. Novagen Brand. Not available through Novagen in Japan.

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**Table 2: Mutation frequency comparison: KOD Hot Start, *PfuTurbo*, *PfuUltra*, and *Taq***

DNA Polymerase	Number of Colonies		Percentage of Mutants Mutation Frequency (%)
	Total	Mutant	
<b>KOD Hot Start</b>	51200	51	
<b><i>PfuUltra</i></b>	49900	53	
<b><i>PfuTurbo</i></b>	65900	164	
<b><i>Taq</i></b>	7000	354	

Mutation Frequency = (Number of mutant colonies/Number of total colonies) × 100%

**Table 3. PCR cycling parameters**

PCR Segment	Number of cycles	DNA Polymerase			
		<i>PfuTurbo</i>	<i>PfuUltra</i> high-fidelity	KOD Hot Start	
Denaturation	1	genomic, λ, or plasmid DNA 95°C, 2 min	genomic, λ, or plasmid DNA 95°C, 2 min	λ or plasmid DNA 94°C, 2 min	genomic DNA 94°C, 2 min
Amplification	25–40	95°C, 30 s Primer (T <sub>m</sub> – 5)°C, 30 s 72°C, 1 min per kbp	95°C, 30 s Primer (T <sub>m</sub> – 5)°C, 30 s 72°C, 1 min (targets < 10 kbp) or 68°C, 2 min per kbp (targets > 10 kbp)	94°C, 15 s Primer [T <sub>m</sub> – (5–10)]°C, 30 s 72°C, 20 s per kbp <sup>1</sup> (targets < 21 kbp)	94°C, 15 s Primer [T <sub>m</sub> – (5–10)]°C, 30 s 68°C, 30 s per kbp <sup>1</sup> (targets < 12 kbp)
Final extension	1	72°C, 10 min	72°C, 10 min	Omit	Omit

<sup>1</sup> Length of the extension step is 10 s per kbp for KOD HiFi. Set up PCR reactions on ice (Novagen TB 320, available at www.novagen.com).

**Table 4. Typical PCR reaction setup**

Component <sup>1</sup>	DNA Polymerase		
	<i>PfuTurbo</i>	<i>PfuUltra</i> high-fidelity	KOD Hot Start
Specific 10X PCR buffer	5 μl	5 μl	5 μl
dNTPs	0.2 mM each	0.2 mM each	0.2 mM each
MgSO <sub>4</sub>	none	none	1 mM
MgCl <sub>2</sub>	about 2 mM <sup>2</sup>	about 2 mM <sup>2</sup>	none
5' primer	0.2–0.5 μM	0.2–0.5 μM	0.3 μM
3' primer	0.2–0.5 μM	0.2–0.5 μM	0.3 μM
Template DNA			
Reverse transcriptase reaction mixture	n/a <sup>3</sup>	n/a <sup>3</sup>	2 μl
Genomic DNA	50–100 ng	50–100 ng	200 ng
λ DNA/plasmid DNA	10 pg–100 ng	0.1–30 ng	1–50 ng
DNA polymerase	2.5 U	2.5 U	1 U
PCR grade water	X μl	X μl	X μl
Total volume	50 μl	50 μl	50 μl

<sup>1</sup> Final reaction concentrations of the components shown are for typical PCR reactions with the indicated DNA polymerases.  
<sup>2</sup> MgCl<sub>2</sub> is included as a component of the 10X PCR buffer for *PfuTurbo* and *PfuUltra* high-fidelity DNA polymerases.  
<sup>3</sup> Data not available.

assay (4, 5). The 4-kbp pMOL21 plasmid, which includes the *bla* gene for ampicillin resistance and the *rpsL*<sup>+</sup> gene for the streptomycin-sensitive phenotype, was used as a template. This study compared mutant-colony production from different DNA polymerases: KOD1 (KOD Hot Start), *Pfu* (*PfuTurbo*<sup>®</sup> and *PfuUltra*<sup>™</sup>), and *Taq*.

The *rpsL* gene of *E. coli* encodes ribosomal protein S12, which binds streptomycin and causes a streptomycin-sensitive phenotype. Mutations in this gene arising from errors during plasmid amplification can result in proteins incapable of binding streptomycin, which leads to streptomycin resistance. Thus, following transformation, the mutation rate can be measured by comparing the number of colonies that grow on amp plus strep plates with the number that grow on amp plates. The number of mutant colonies resulting from each of the four DNA polymerases examined (Table 2) demonstrates comparable mutation frequencies between KOD Hot Start and *PfuUltra*, two-fold higher mutation frequency using *PfuTurbo*, and 500-fold greater mutation frequency using *Taq*.

## Summary

We have demonstrated that KOD Hot Start DNA Polymerase is a superior proof-reading DNA polymerase capable of generating high yields of PCR amplicons and exhibiting very high fidelity, comparable to that of *PfuUltra* DNA Polymerase. By using KOD Hot Start DNA Polymerase in place of *Pfu* or *Taq* DNA polymerase in a standard PCR protocol, better results can be achieved in shorter times (Tables 3 and 4).

## REFERENCES

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Product	Size	Cat. No.
KOD Hot Start DNA Polymerase	200 U 5 × 200 U	71086-3 71086-4
[includes KOD Hot Start DNA Polymerase (1.0 U/μl), 10X PCR Buffer for KOD Hot Start DNA Polymerase, 25 mM MgSO <sub>4</sub> , and dNTP Mix (2 mM each)]		
pSTBlue-1 Perfectly Blunt <sup>®</sup> Giga Cloning Kit	20 rxn 40 rxn	71229-3 71229-4
[includes Blunt Vector, Positive Control Insert, End Conversion Mix, T4 DNA Ligase, Nuclease-free Water, NovaBlue GigaSingles <sup>™</sup> Competent Cells, SOC Medium, and Test Plasmid]		
pSTBlue-1 Perfectly Blunt Cloning Kit	20 rxn 40 rxn	70191-3 70191-4
[includes Blunt Vector, Positive Control Insert, End Conversion Mix, T4 DNA Ligase, Nuclease-free Water, NovaBlue Singles <sup>™</sup> Competent Cells, SOC Medium, and Test Plasmid]		