

KOD DNA Polymerase

KOD DNA Polymerase

250 U

71085-3

About the Kits

Description

KOD DNA Polymerase is a recombinant form of *Thermococcus kodakaraensis* KOD1 DNA Polymerase (1). KOD is a high fidelity thermostable polymerase amplifying target DNA up to 6 kbp with superior accuracy and yield (2). The enzyme's 3'→5' exonuclease dependent proofreading activity results in a lower mutation frequency than any other commercially available DNA polymerase. The elongation rate and processivity are 5 times and 10–15 times higher, respectively, than *Pfu* DNA polymerase, resulting in highly accurate and robust yield in a short reaction time. KOD DNA Polymerase produces blunt-ended DNA products suitable for cloning with the Novagen Perfectly Blunt® and LIC Systems and is compatible with site-directed mutagenesis protocols.

Enzyme	KOD 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* (mutation frequency)	0.0035	0.0039	0.013
Elongation rate (bases/second)	106–138	25	61
Processivity** (nucleotide bases)	> 300	< 20	not determined

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

** Processivity is defined as the number of nucleotides that can be extended in one catalytic reaction by one DNA polymerase molecule.

© 2007 EMD Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany. All rights reserved. Perfectly Blunt® and the Novagen® name are registered trademarks of EMD Biosciences, Inc. KOD Polymerases are manufactured by TOYOBO and distributed by EMD Biosciences, Inc., Novagen brand. KOD DNA Polymerase is licensed under U.S. Patent No. 5,436,149 owned by Takara Shuzo, Co., Ltd.

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as the patented 5' Nuclease Process claims in US Patents Nos. 5,210,015 and 5,487,972), no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a few or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

USA and Canada
Tel (800) 526-7319
novatech@novagen.com

Germany
Tel 0800 100 3496
techservice@merckbiosciences.de

United Kingdom and Ireland
UK Freephone 0800 622935
Ireland Toll Free 1800 409445
customer.service@merckbiosciences.co.uk

All Other Countries
Contact Your Local Distributor
www.novagen.com
novatech@novagen.com

A Brand of EMD Biosciences, Inc., an Affiliate of Merck KGaA, Darmstadt, Germany
www.novagen.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

Components

- 250 U KOD DNA Polymerase (2.5 U/ μ l in 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.1% Nonidet P-40, 0.1% Tween-20, pH 8.0)
- 1 ml 10X Buffer #1 for KOD DNA Polymerase (10X = 1.2 M Tris-HCl, 100 mM KCl, 60 mM $(\text{NH}_4)_2\text{SO}_4$, 1% Triton X-100, 0.01% BSA, pH 8.0)
- 1 ml 10X Buffer #2 for KOD DNA Polymerase (10X = 1.2 M Tris-HCl, 100 mM KCl, 60 mM $(\text{NH}_4)_2\text{SO}_4$, 1% Triton X-100, 0.01% BSA, pH 8.8)
- 1 ml 25 mM MgCl_2
- 1 ml dNTPs (2 mM each)

Unit definition

One unit is defined as the amount of enzyme that will catalyze the incorporation of 10 nmol dNTP into acid insoluble form in 30 minutes at 75°C in a reaction containing 20 mM Tris-HCl (pH 7.5 at 25°C), 8 mM MgCl_2 , 7.5 mM DTT, 50 μ g/ml BSA, 150 μ M each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [^3H] dTTP) and 150 μ g/ml activated calf thymus DNA.

Storage

Store all components at -20°C.

KOD DNA Polymerase Protocol

KOD DNA Polymerase and buffers are a unique PCR system. The following procedure is designed for use with the components provided in the KOD DNA polymerase kit. Using reaction components or protocols designed for any other DNA polymerase may result in poor amplification.

Reaction conditions listed below will provide satisfactory amplification for most primer/template combinations. Guidelines and troubleshooting sections provide details for optimizing reaction conditions. Remember to include a negative control reaction lacking only template; inclusion of a positive control reaction using a template known to amplify with the primers may also be helpful. Concentrations of enzyme, MgCl₂, template, and primers can be varied to optimize the reaction.

Standard reaction setup

Component	Volume	Final Concentration
10X Buffer for KOD DNA Polymerase	5 µl	1X
25 mM MgCl ₂	3 µl	1.5 mM
dNTPs (2 mM each)	5 µl	0.2 mM (each)
PCR Grade Water	27.6 µl	
Sense (5') Primer (5 pmol/µl)	4 µl	0.4 µM
Anti-Sense (3') Primer (5 pmol/µl)	4 µl	0.4 µM
Template DNA ^a	1 µl	
KOD DNA Polymerase (2.5 U/µl)	0.4 µl	0.02U/µl
Total reaction volume	50 µl	

Note: To prevent degradation of the primers, add the polymerase and primers last and keep the reaction on ice until ready for thermal cycling.

^a See Template DNA section on page 4.

Cycling conditions

Temperature and time

The following table allows for primer extension that occurs during temperature ramping between steps.

Phage and plasmid DNA templates

Cycling parameters	0.5 kbp target DNA	1–2 kbp target DNA	3–4 kbp target DNA	5–6 kbp target DNA
1. Denature	15 s 98°C	15 s 98°C	15 s 98°C	30 s 94°C
2. Anneal	1–30 s 68°C	2 s lowest primer T _m °C	5 s lowest primer T _m °C	30 s lowest primer T _m °C
3. Extend	none	20 s 72°C	40 s 72°C	60 s 72°C
4. Repeat steps 1-3.	20–30 cycles. For more information see cycle number on page 4.			

Genomic DNA templates

Cycling parameters	Up to 2 kbp
1. Denature	15 s 95°C
2. Anneal	30 s lowest primer T _m °C
3. Extend	30–60 s 72°C
4. Repeat steps 1-3.	30–40 cycles

cDNA templates

Cycling parameters	Up to 2 kbp
1. Denature	20 s 98°C
2. Anneal	30 s 68°C
3. Extend	none
4. Repeat steps 1-3.	30 cycles

Cycle number

The number of cycles (steps 1 through 3 in the previous table) required to generate a PCR product will depend on the source and amount of starting template in the reaction, as well as the efficiency of the PCR. In general, 20–40 cycles will be adequate for a wide range of templates. It is common to use fewer cycles when amplifying targets from plasmids (i.e., subcloning) where a high number of copies of template is easily attained, as this reduces the chance of amplifying a mutation. A higher number of cycles (e.g., 40) may be necessary when amplifying from genomic DNA since the target sequence will be in low abundance.

Additional Guidelines**Primers**

Primer design is critical for successful PCR amplification. Because KOD DNA polymerase exhibits strong 3'→5' exonuclease activity, primers should be at least 21 bases of 3' end complementary to the target sequence. G/C content of the primers should be 40–60%. Primer melting temperature (T_m) is defined as the temperature at which one half of the DNA duplex will dissociate to become single stranded. Some primer molecules will anneal as the temperature approaches the T_m of a primer, as a result PCR amplifications are usually successful over a range of annealing temperatures. Primer pairs with similar T_m values usually result in better amplifications because annealing and extension are better synchronized. If melting temperatures of a primer pair differ by more than 5°C, increasing the length of the lower- T_m primer will reduce the difference. Primers for two-step cycling programs should be designed with a high T_m value to ensure proper annealing and extension at the same temperature.

There are several methods for determining the T_m of a primer. The nearest-neighbor method (3) using 50 mM monovalent salt is one method for T_m prediction. Unlike other methods, the nearest-neighbor method takes into account the primer sequence and other variables such as salt and DNA concentration. The T_m can also be calculated with the % GC method (4). The most general method of calculating the T_m is based on the number of adenine (A), thymidine (T), guanidine (G) or cytosine (C) bases where $T_m(^{\circ}\text{C}) = 2(N_A + N_T) + 4(N_G + N_C)$.

Primer T_m values reported by manufacturers may vary by 5 to 10°C depending on the calculation method used. In addition, the exact T_m for a given primer in a reaction may be affected by DNA concentrations (primer and template), mono and divalent ion concentrations, dNTP concentration, presence of denaturants (e.g., DMSO), and nucleotide modifications. Therefore, an optimal primer annealing temperature should be determined empirically.

When receiving oligonucleotides from the manufacturer, prepare primer stocks at 100 pmol/μl (100 μM) in TE and store them at –20°C. To set up KOD reactions, dilute enough of each primer stock 20-fold (5 μM) to add 4 μl per reaction.

Template DNA

The optimal amount of starting template may vary depending on the template quality. Amplification is generally more difficult when there are few copies of the target DNA such as genomic DNA or cDNA, as compared to plasmid or phage DNA. In general the suggested amount of template DNA for amplification is 0.006–6 ng for plasmid or phage DNA and up to 12 ng genomic DNA or cDNA template. Using too much template in the PCR reaction can result in failed reactions since template denaturation is concentration dependent. At high concentrations of DNA, denaturation is less efficient.

Long target DNA

Amplification of long target DNA (5–6 kbp) using PCR Buffer #2 may enhance the quality and quantity of the PCR product. Also, the addition of DMSO to 2–10% v/v final concentration may reduce secondary structure of the template DNA and increase yield.

GC-rich templates

The addition of DMSO to 2–10% final concentration may decrease template secondary structure and increase yield. Final DMSO concentrations of less than 5% v/v have no effect on fidelity (5, 6). The effect of DMSO above 5% v/v on enzyme fidelity has not yet been determined.

Genomic DNA

For genomic DNA templates, amplification using PCR Buffer #2 may enhance the quality and quantity of the PCR product. Increasing the concentration of dNTPs to 0.3 mM may also enhance the amplification of genomic DNA targets. Although a greater concentration of dNTPs in the PCR reaction can increase yield, it also can reduce specificity and fidelity.

Unpurified templates

Crude cell lysates, PCR products, plaques, and colonies can serve as template for PCR. Limit the volume of unpurified templates to reduce inhibition of the reaction.

Extension time

Since KOD is highly processive, long extension times may cause smearing. A two step cycling reaction, that combines the annealing and extension steps, is often used for short target DNA from template DNA in multiple copies (plasmid and phage DNA). Although the extension time can be increased for longer target DNA, targets above 6 kbp are difficult to amplify due to the strong 3'→5' exonuclease activity.

Extension temperature

The extension temperature can be increased to 74°C to increase yield.

PCR Buffer and dNTP concentration

PCR Buffer #1 is appropriate for most applications. Amplification of long target DNA (5–6 kbp) and genomic DNA using PCR Buffer #2 may enhance the quality and quantity of the PCR product. Increasing the concentration of dNTPs to 0.3 mM may enhance the amplification of genomic DNA targets. Although a greater concentration of dNTPs in the PCR reaction can increase yield, it also can reduce specificity and fidelity.

Two-step PCR

In two-step PCR, annealing and extension can be carried out at the same temperature. Primers for two-step cycling programs should be designed with high T_m values (> 65°C) to ensure proper annealing and extension at the same temperature. Initially try an annealing/extension temperature equal to the lowest T_m of the primer pair. Since polymerase speed is slower at 68°C, increase the annealing/extension time by 5 s/kbp during two-step cycling.

Optimization

When optimizing PCR reactions, it is best to change only one parameter at a time. The use of DMSO at 5% v/v final often improves a suboptimal PCR.

Troubleshooting

Symptom	Possible cause	Solution
No PCR product	Target size too large	Use a smaller target size. KOD amplifies up to 2 kbp genomic DNA and up to 6 kbp plasmid and phage DNA targets.
Smear instead of distinctive DNA band on agarose gel	Reactions were not set up on ice	The reaction should be set up on ice and the KOD should be added last to the PCR reaction mix to prevent degradation of primers and template.
	Suboptimal PCR conditions	Decrease annealing and extension times according to the table on page 3. KOD extension rate is faster than other thermostable polymerases and longer extension times can cause smearing.
Low yield	High GC content	Add DMSO to a final concentration of 2–5%. DMSO concentration less than 5% does not change enzyme fidelity.
	Long target/genomic DNA	Using PCR Buffer #2 may enhance the quality and quantity of the PCR product. Add DMSO to a final concentration of 2–5%. DMSO concentration less than 5% does not change enzyme fidelity.
	Low amount of template	For plasmid or phage DNA 0.006–6 ng of template is adequate. Genomic and cDNA templates may require up to 12 ng.

Applications

This section lists references for applications with KOD DNA Polymerase. Please visit www.novagen.com/KOD for the latest information.

Application	Reference
Construction of knock-out targeting vector	Kim, T. S., Maeda, A., Maeda, T., Heinlein, C., Kedishvili, N., Palczewski, K., and Nelson, P. S. (2005) <i>J. Biol. Chem.</i> 280, 8964–8704.
Gene cloning	Herrin, B. R., Groger, A. L., and Justement, L. B. (2005) <i>J. Biol. Chem.</i> 280, 3507–3515.
	Ikehara, Y., Ikehara, S. K., and Paulson, J. C. (2004) <i>J. Biol. Chem.</i> 279, 43117–43125.
	Momose, F., Basler, C. F., O'Neill, R. E., Iwamatsu, A., Palese, P., and Nagata, K. (2001) <i>J. Virol.</i> 75, 1899–1908.
	Matsuura, A., Kinebuchi, M., Chen, H., Katabami, S., Shimizu, T., Hashimoto, Y., Kikuchi, K., and Sato, N. (2000) <i>J. Immunol.</i> 164, 3140–3148.
	Kitagawa, M., Hatakeyama, S., Shirane, M., Matsumoto, M., Ishida, N., Hattori, K., Nakamichi, I., Kikuchi, A., Nakayama, K., and Nakayama, K. (1999) <i>EMBO J.</i> 18, 2401–2410.
	Nakashima, N., Noguchi, E., and Nishimoto T. (1999) <i>Genetics</i> 152, 853–867.
Genomic DNA cloning	Nisole, S., Lynch, C., Stoye, J. P., and Yap, M. W. (2004) <i>Proc. Natl. Acad. Sci. USA.</i> 101, 13324–13328.
Second strand cDNA synthesis	Sasaki, Y., Casola, S., Kutok, J. L., Rajewsky, K., and Schmidt-Supprian, M. (2004) <i>J. Immunol.</i> 173, 2245–2252.
	Yazaki, K., Shitan, N., Takamatsu, H., Ueda, K., and Sato, F. (2001) <i>J. Exp. Bot.</i> 52, 877–879.
Synthetic gene synthesis	Wu, G., Wolf, J. B., Ibrahim, A. F., Vadasz, S., Gunasinghe, M., and Freeland, S. (2006) <i>J. Biotechnol.</i> 124, 496–503.

References

1. Nishioka, M., Mizuguchi, H., Fujiwara, S., Komatsubara, S., Kitabayashi, M., Uemura, H., Takagi, M. and Imanaka, T. (2001) *J. Biotechnol.* **88**, 141–149.
2. Takagi, M., Nishioka, M., Kakihara, H., Kitabayashi, M., Inoue, H., Kawakami, B., Oka, M. and Imanaka, T. (1997) *Applied and Environmental Microbiology* **63**, 4504–4510.
3. Breslauer, K. J., Frank, R., Blocker, H. and Marky, L. A. (1986) *Proc. Natl. Acad. Sci.* **83**, 3746–3750.
4. Howley, P. M., Israel, M. A., Law, M. F. and Martin, M. A. (1979) *J. Biol. Chem.* **254**, 4876–4883.
5. Cheng, S., Fockler, C., Barnes, W. M. and Higuchi, R. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 5695–5699.
6. Winship, P. R. (1989) *Nucleic Acids Res.* **17**, 1266.