KOD DNA Polymerase

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About the Kits

KOD DNA Polymerase	250 U	71085-3

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	*Pfu* DNA Polymerase	*Taq* DNA Polymerase
Species	*Thermocccus kodakaraensis*	*Pyrococcus furiosus*	*Thermus aquaticus 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.

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 $MgCl_2$, 0.5 mM DTT, 50 µg/ml BSA, 150 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [^{3}H] dTTP) and 150 µg/ml activated calf thymus DNA.

Components

- 250 U KOD DNA Polymerase (2.5 U/µl 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 $(NH_4)_2SO_4$, 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 $(NH_4)_2SO_4$, 1% Triton X-100, 0.01% BSA, pH 8.8)
- 1 ml 25 mM $MgCl_2$
- 1 ml dNTPs (2 mM each)

Storage

Store all components at –20°C.

Standard Protocol

In many cases, the standard reactions described below will provide satisfactory amplification. 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, $MgCl_2$, template and primers can be varied to optimize the reaction.

1. For each 50 µl reaction, assemble the following in a 0.5 ml PCR tube **on ice** just prior to use.

28.6 µl	PCR Grade Water
5 µl	10X Buffer#1 For KOD DNA Polymerase
5 µl	dNTPs (final concentration 0.2 mM)
2 µl	$MgCl_2$ (final concentration 1 mM)
1 µl	template DNA
4 µl	5' primer (5 pmol/µl, final concentration 0.4 µM)
4 µl	3' primer (5 pmol/µl, final concentration 0.4 µM)
0.4 µl	KOD DNA Polymerase (2.5 U/µl)
50 µl	total volume

Note: The addition of 2–5% DMSO can improve amplification with GC-rich or long templates and will not decrease the fidelity (3, 4).

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

2. Mix gently and centrifuge briefly to bring reaction components to the bottom of the tube. If necessary, add mineral oil to cover the reaction, cap the tubes, and place in the thermal cycler.
3. The following are several thermal cycling program options. Note that the choice of primers affects the annealing temperature. In general use an annealing temperature 5°C below the T_m (melting temperature) of the primers as a starting point.

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
Denature	15 sec 98°C	15 sec 98°C	15 sec 98°C	30 sec 94°C
Anneal	1–30 sec 68°C	2 sec (T_m-5)°C	5 sec (T_m-5)°C	30 sec (T_m-5)°C
Extend	none[a]	20 sec 72°C	40 sec 72°C	60 sec 72°C
No. cycles	25	25	25–30	30

Genomic DNA templates

Cycling parameters	Up to 2 kbp
Denature	15 sec 95°C
Anneal	30 sec (T_m-5)°C
Extend	30–60 sec 72°C
No. cycles	30

cDNA templates

Cycling parameters	Up to 2 kbp
Denature	20 sec 98°C
Anneal	30 sec 68°C
Extend	none[a]
No. of cycles	30

[a] Primers used for two-step cycling programs must have an appropriate T_m value to anneal at 68°C (see *Primers*).

4. To analyze the reaction products, remove a 10 µl sample from beneath the oil overlay and add to appropriate loading buffer. Load and run an agarose gel containing 0.5 µg/ml ethidium bromide and visualize the bands under UV illumination.

Additional Guidelines

Primers

Primer design is important for successful PCR amplification. The G/C content should be approximately 40–60% (a G/C content of greater than 60% may require a higher denaturation temperature or a longer denaturation time). Primer pairs should exhibit similar melting temperatures (T_m). 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. In general, use an annealing temperature that is 5–10°C less than the lowest T_m of the primer pair as a starting point.

There are several methods for determining the T_m of a primer. The nearest-neighbor method (5) using 50 mM monovalent salt is recommended for accurate 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 (6). 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; $T_m(°C) = 2(N_A + N_T) + 4(N_G + N_C)$. However, the exact T_m of a given primer may be affected by DNA concentration, presence of denaturants (e.g., DMSO), and nucleotide modifications (e.g., biotin, fluorescent dyes, etc.).

Template DNA

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. For plasmid or phage DNA, 0.006–6 ng is adequate, whereas genomic and cDNA templates may require up to 12 ng.

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.

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 kpb 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 2. 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 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 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.merck4biosciences.com for more 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) *J. Biol. Chem.* 280, 8964–8704.
Gene cloning	Herrin, B. R., Groger, A. L., and Justement, L. B. (2005) *J. Biol. Chem.* 280, 3507–3515.
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Genomic DNA cloning	Nisole, S., Lynch, C., Stoye, J. P., and Yap, M. W. (2004) *Proc. Natl. Acad. Sci. USA.* 101, 13324–13328.
Second strand cDNA synthesis	Sasaki, Y., Casola, S., Kutok, J. L., Rajewsky, K., and Schmidt-Supprian, M. (2004) *J. Immunol.* 173, 2245–2252.
	Yazaki, K., Shitan, N., Takamatsu, H., Ueda, K., and, Sato, F. (2001) *J. Exp. Bot.* 52, 877–879.
Synthetic gene synthesis	Wu, G., Wolf, J. B., Ibrahim, A. F., Vadasz, S., Gunasinghe, M., and Freeland, S. (2006) *J. Biotechnol*. 124, 496–503.

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