KOD XL DNA Polymerase

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About the Kits

KOD XL DNA Polymerase	250 U	71087-3
	1250 U	71087-4

Description

KOD XL DNA Polymerase is an optimized mixture of KOD DNA Polymerase and a mutant form of KOD that is deficient in 3'→5' exonuclease activity. This enzyme mixture is designed for accurate and rapid amplification of complex, GC-rich, and long (up to 30 kbp) target DNA (1). It can also be used for incorporation of derivatized dNTPs in PCR amplicons (2, 3). KOD XL DNA Polymerase produces blunt ends and 3' single nucleotide overhangs suitable for cloning in AccepTor™ or T-vector methods, as well as the Novagen's Perfectly Blunt® and LIC Systems.

Unit definition: One unit is defined as the amount of enzyme that will catalyze the incorporation of 10 nmol of dNTP into acid insoluble form in 30 min 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 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 or 5 × 250 U — KOD XL DNA Polymerase (2.5 U/µl in 50 mM KCl, 50 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, 0.1% Nonidet P–40, 0.1% Tween® 20, pH 8.0)
- 1.2 ml or 5 × 1.2 ml — 10X PCR Buffer for KOD XL DNA Polymerase
- 1 ml or 5 × 1 ml — dNTPs (2 mM each)

Storage

Store all components in a constant-temperature freezer at –20°C.

KOD XL DNA Polymerase Protocol

KOD XL DNA Polymerase and buffer are a unique PCR system. The following procedure is designed for use with the components provided in the KOD XL 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.

Standard reaction setup

Component	Volume	Final Concentration
10X Buffer for KOD XL DNA Polymerase	5 µl	1X
dNTPs (2 mM each)	5 µl	0.2 mM (each)
PCR Grade Water	X µl	
Sense (5') Primer (5 pmol/µl)	2 µl	0.2-0.4 µM
Anti-Sense (3') Primer (5 pmol/µl)	2 µl	0.2-0.4 µM
Template DNA[a]	Y µl	
KOD XL DNA Polymerase (2.5 U/µl)	1 µl	0.05 U/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.

Cycling parameters	≤ 2 kbp target DNA	2–6 kbp target DNA	6–10 kbp target DNA	10–12 kbp target DNA
1. Denature	30 s 94°C	30 s 94°C	30 s 94°C	30 s 94°C
2. Anneal	5 s (T_m-5)°C	5 s (T_m-5)°C	5 s (T_m-5)°C	5 s (T_m-5)°C
3. Extend	30–60 s 70–74°C	30–60 s/1 kbp 70–74°C	5–6 min 70–74°C	8 min 70–74°C
4. Repeat steps 1–3	25–30 cycles. For more information see "Cycle number" in the following section.			
5. Final Extension	10 min 74°C	10 min 74°C	10 min 74°C	10 min 74°C

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, 25–30 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., 30) 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. The primers should be 21 bases long or longer for best results with 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.

There are several methods for determining the T_m of a primer. The nearest-neighbor method (4) 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 (5). 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 $T_m(°C) = 2(N_A + N_T) + 4(N_G + N_{C.})$.

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 2 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 1–50 ng phage DNA, 1–50 ng plasmid DNA, 100–1000 ng genomic DNA, or 2 µl of a reverse transcription reaction. To start, use 2.5 ng of phage/plasmid DNA for 25 cycles and 100 ng of genomic DNA for 30 cycles with the cycling programs listed on page 3. 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.

Plasmid templates

For subcloning, amplify from 10 ng of plasmid template and reduce the number of cycles to 20–25.

GC-rich templates

The addition of DMS0 to 2–5% final concentration may decrease template secondary structure and increase yield. Final DMSO concentrations of less than 5% v/v have no effect on fidelity (6, 7).

Long target DNA

For target DNA over 8 kbp, increasing the dNTP concentration to 0.35 mM may reduce the appearance of multiple bands. Also, the addition of DMSO to 2–10% v/v final concentration may reduce secondary structure of the template DNA and increase yield.

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.

Note: If the target DNA is smeared, reduce the units of KOD XL DNA polymerase and/or decrease the extension time.

Troubleshooting

Symptom	Possible cause	Solution
No PCR product	PCR primers are not long enough	Use primers longer than 21 bases.
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 XL should be added last to the PCR reaction mix to prevent degradation of the primers and template.
	Suboptimal PCR conditions	Reduce the units of KOD XL DNA Polymerase and/or decrease the extension time.
Low yield	High GC content/long target DNA	Add DMSO to a final concentration of 2–5%. DMSO concentration less than 5% does not change enzyme fidelity.
Multiple Bands	Long target DNA	For target DNA over 8 kbp, increasing the dNTP concentration to 0.35 mM may reduce the appearance of multiple bands.

Applications

This section lists references for applications with KOD XL DNA Polymerase.

Application	**Reference**
Construction of combinatorial protein libraries by random multi-recombinant PCR	Tsuji, T., Onimaru, M. and Yanagawa, H. (2001) *Nucleic Acids Res.* **29**, E97
Gene Cloning	Kim, T. S., Maeda, A., Maeda, T., Heinlein, C., Kedishvili, N., Palczewski, K., and Nelson, P. S. (2005) *J. Biol. Chem.* **280**, 8964–8704.
	Ogawara, Y., Kishishita, S., Obata, T., Isazawa, Y., Suzuki, T., Tanaka, K., Masuyama, N. and Gotoh, Y. (2002) *J. Biol. Chem.* **277**, 21843–21850.
Genotyping, crude genomic DNA preparation from clinical samples	Mitsumori, K., Onodera, H., Shimo T., Yasuhara, K., Takagi, H., Koujitani, T., Hirose, M., Maruyama, C., and Wakana S. (2000) *Carcinogenesis* **21**, 1039–1042.
	Sasagawa, T., Basha, W., Yamazaki, H. and Inoue, M. (2001) *Cancer Epidemiol. Biomarkers Prev.* **10**, 45–52.
Incorporation of derivatized dNTPs	Sawai, H., Ozaki-Nakamura, A., Mine, M., and Ozaki, H. (2002) *Bioconjug. Chem.* **13**, 309–319.
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Multiplex colony-direct PCR	Okitsu, T., Suzuki, R., and Yamai, H. (2001) i*nNovations* **17**, 11.
Second strand cDNA synthesis	Tabuchi, I., Soramoto, S., Suzuki, M., Nishigaki, K., Nemoto, N., and Husimi, Y. (2002) *Biol. Proced. Online* **4**, 49–54.
	Tanaka T., Isono, T., Yoshiki, T., Yuasa, T., and Okada, Y. (2000) *Cancer Res*. **60**, 56–59.
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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