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BioSmart KOD Polymerase

Cat Number: BSKOD01

Unit: 2.5U/μL, 100μL/vial

Sources: *Thermococcus kodakaraensis*., recombinant modified

Product Description

KOD DNA Polymerase is a high fidelity thermostable polymerase amplifying target DNA up to 6 kbp with superior accuracy and yield. The enzyme exhibits 3'→5'exonuclease dependent proofreading activity and results in a lower PCR mutation frequency. The elongation rate and processivity are 5 times and 10-15 times higher respectively, than Pyrococcus furiosus Pfu DNA Polymerase, that resulting in highly accurate and robust yield in a short reaction time.

Application: Broad range amplicon PCR, Suitable for cloning and site-directed mutagenesis protocols

Unit definition: One unit is defined as the amount of enzyme that will catalyze the incorporation of 10 nmole dNTP into acid insoluble form in 30 minutes at 75℃.

Components

- 250 U KOD DNA Polymerase (2.5U/μl in 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1mM EDTA, 50% Glycerol, 0.1% NP-40, 0.1% Tween 20, pH 8.0)
- 1mL 10X Buffer #1 for KOD DNA Polymerase (1.2M Tris-HCl, 100 mM, KCl, 60 mM $(NH_4)_2SO_4$, 1% Triton X-100, 0.01% BSA, pH 8.0)
- 1mL 10X Buffer #2 for KOD DNA Polymerase (1.2M Tris-HCl, 100 mM, KCl, 60 mM $(NH_4)_2SO_4$, 1% Triton X-100, 0.01% BSA, pH 8.8)
- 1mL 25 mM $MgCl_2$

Storage: store all components at-20℃

Protocol

Concentrations of enzyme, $MgCl_2$, template and primers can be varied to optimize the reaction.

1. Set up each reaction as follows (Keep on ice)

Component	50 µl reaction	Final Concentration
10X KOD Reaction Buffer	5µl	1x
Forward Primer	Variable	0.1-1.0 µM
Reverse Primer	Variable	0.1-1.0 µM
10mM dNTPs	Variable (dependent PCR product size	200-300 µM of each dNTP
Template DNA	Variable	<1 µg
KOD DNA Polymerase	0.5µl	1-2units/50 µl PCR
ddH2O	to 50ul	

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

2. Mix gently and centrifuge briefly to bring reaction components to the bottom of the tube.
3. Perform PCR using the recommended thermal cycling conditions outlined below:

Cycling parameters	Temperature	Time
Initial Denaturation	95℃	5min
Denaturation (30 cycles)	95℃	15-30 sec
Annealing (30 cycles)	48-68℃	15-60 sec
Extension (30 cycles)	68 or 72℃	0.5min/kb
Final Extension	72℃	5min
Hold	4℃	

Note: The following are several thermal cycling program options that the choice of primers affects the annealing temperature. In general use an annealing temperature 5℃ below the Tm of the primers as a starting point.

Mg^{2+} and additives

The optimal Mg^{2+} concentration of 1mM empirically, will generate satisfactory amplification of most amplicons. Amplification of some cases, reactions may be improved with additives, like DMSO.

Notes

1. PCR Buffer #1 is appropriate for most applications. Amplification of long target DNA (>6 kbp) and genomic DNA using PCR buffer #2 may enhance the quality and quantity of the PCR product.

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