KOD DNA Polymerase

Cat. Number: KOD

Storage: Store at -20°C.

Unit: 5U/uL, 100uL/vial

Sources: *Thermococcus kodakaraensis.*, recombinant modified

Application

Broad range amplicon PCR

Description

KOD DNA Polymerase is a high fidelity thermostable DNA polymerase that exhibits 3'→ 5' exonuclease (proofreading) activity and results in a lower PCR mutation frequency.

KOD DNA Polymerase exhibits excellent processivity and elongation capability, showing a five-fold higher extension rate (100-130 nucleotides/second) and 10-15-fold higher processivity (>300 bases) than that from Pyrococcus furiosus (Pfu DNA polymerase). The elongation rate of this enzyme is approximately 2 times higher than that of Taq DNA polymerase.

Unit Definition

One unit is defined as the amount of enzyme that synthesizes 1 nmol of DNA within 3 minutes at 72°C.

Unit Assay Conditions

1X KOD Reaction Buffer, 125 µM dNTPs and 15 nM primed M13 DNA.

1X KOD Reaction Buffer

10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 10% DMSO.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween® 20, 50% Glycerol.

Molecular Weight

Theoretical: 90,000 daltons.

Mutation Frequency

(Number of mutant base pairs / Number of total base pairs) X 100% = 0.02%

Protocol

1. We recommend assembling all reaction components on ice and quickly transferring the reaction to a thermocycler preheated to the denaturation temperature (95°C).

2. 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	1ul	200 µM of each dNTP
Template DNA	variable	<1 ug
KOD DNA Polymerase	0.5µl	2.5 units/50 µl PCR
ddH2O	to 50µl	

3. Gently mix the solution a few times and spin down.

4. Perform PCR using the recommended thermal cycling conditions outlined below:
(For maximum yield and specificity, temperatures and cycling times should be optimized for each new template target or primer pair)

STEP		TEMP	TIME
Initial Denaturation		95ºC	5min
Denaturation	30 cycles	95ºC	15-30sec
Annealing		45-68ºC	15-60sec
Extension		72ºC	1min/kb
Final Extension		72ºC	5min
Hold		4ºC	

*Annealing temperature: 5°C below the Tm of your primers.

Mg2+ and additives:

The optimal Mg2+ concentration of 1.5 mM empirically, as provided in the 1X KOD Reaction Buffer, will generate satisfactory amplification of most amplicons. However, in some difficult targets, Mg2+ can be improved by increasing 0.5-1.5 mM. Amplification of some cases, reactions may be improved with additives, like DMSO.