Kod Plus DNA Polymerase

Catalog #	Pack size	Price(€)
ZP02033	200U	80.00
ZP02034	500U	150.00

Description:

DNA polymerase from Thermococcus kodakaraensis KOD is one of the most efficient thermostable PCR enzymes exhibiting higher accuracy and elongation velocity than any other commercially available DNA polymerase.The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'---->3' direction. The KOD DNA Polymerase also exhibits 3'--->5'exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5'--->3' exonuclease activity.

Source:

KOD DNA Polymerase (native) - Thermococcus kodakaraensis cells.KOD DNA Polymerase (recombinant) - E.coli cells with a cloned pol gene from Thermococcus kodakaraensis.

Molecular Weight:

91 kDa monomer

Features:

- High fidelity than other normal DNA polymerase
- Higher processivity.
- Higher yield in short time

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 (pH7.5 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.

Applications:

- All PCR* amplification which demands high fidelity.

Add: Floor 2,Building A,328#, Wuhe Road,Minhang District,Shanghai,201109,China
Tel: +86-21-54460832 Web: www.synthesisgene.com
Fax:+86-21-54460831 E-mail: master@shinegene.org.cn

- Primer extension.
- High fidelity PCR for cloning into blunt-ended vectors.
- Site-directed mutagenesis.

10X Reaction Buffer (without Mg^{2+}):

1 ml 10X Buffer : 1.2 M Tris-HCl, 100 mM KCl, 60 mM $(NH_4)_2SO_4$, 1% Triton X-100, 0.01% BSA, pH8.0 and pH8.8)

Storage Buffer and Concentration:

1U/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, pH8.0

Storage:

-20℃.

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.

I. For each 50 μl reaction, assemble the following in a 0.2 ml PCR tube on ice just prior to use.

30.0 μl	PCR Grade Water
5 μl	10X Reaction Buffer
4ul	25mM MgSO4(final concentration 2.0-3.0mM)
1 μl	dNTP Mixture (10 mM each, final concentration 0.2 mM)
1 μl	Template DNA(10-500pg)
4 μl	5' primer (5 pmol/μl, final concentration 0.4 μM)
4 μl	3' primer (5 pmol/μl, final concentration 0.4 μM)
1 μl	*Kod plus* DNA Polymerase (1U/μl)
50 μl	**total volume**

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

II. 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.

III. 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℃ below the T_m (melting temperature) of the primers as a starting point.

Phage and plasmid DNA templates

Cycling Parameters	**1-2 kb Target DNA**	**3-4 kb Target DNA**	**5-6 kb Target DNA**	**7-10 kb Target DNA**
Predenature	2 min 94℃	2 min 94℃	2 min 94℃	2 min 94℃
Denature	20 sec 94℃	20 sec 94℃	15 sec 94℃	15 sec 94℃
Anneal	20 sec (T_m-5) ℃	1Kbp/min 68℃	1Kbp/min 68℃	1Kbp/min 68℃
Extend	1-2 min 72℃	none	none	none
No. Cycles	25-30	25-30	30	30

Genomic DNA templates **cDNA templates**

Cycling Parameters	**Up to 2 kb**		**Cycling Parameters**	**Up to 2 kb**
Predenature	2 min 94℃		Predenature	2 min 94℃
Denature	15 sec 94℃		Denature	15 sec 94℃
Anneal	30 sec (T_m-5) ℃		Anneal	1Kbp/min 68℃
Extend	1-2 min 72℃		Extend	None
No. Cycles	30		No. Cycles	30

IV. 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.

Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add：Floor 2,Building A,328#, Wuhe Road, Shanghai 201109,

Tel：+86-21-54460832

Fax:+86-21-54460831

E-mail:shinegene@vip.163.com

Website：www.synthesisgene.com