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DNA Polymerase I, Large (Klenow) Fragment

Product Number: PC18

Shipping and Storage

Storage at -20℃.

Component

Component	PC18-200U	PC18-1KU	PC18-5KU
Klenow Fragment (5U/μL)	40μL	200μL	1mL
Reaction Buffer (10X)	200μL	1mL	5mL

Description

The Klenow Fragment, also known as the Klenow fragment, is a large fragment of Escherichia coli DNA polymerase I (E. coli DNA polymerase I). It retains the 5'→3' polymerase activity and 3'→5' exonuclease activity of DNA polymerase I but lacks the complete 5'→3' exonuclease activity of the full Klenow enzyme. The 3'→5' exonuclease activity of the Klenow Fragment ensures the fidelity (proofreading) of DNA synthesis.

Application

Fill in at the 5 'overhang end of double stranded DNA; Flattening (also known as flattening) the 3 'overhang of double stranded DNA; 5 'protruding end mark; Random primer method for DNA labeling; Sanger deoxygenation method for DNA sequencing; The synthesis of the second strand of cDNA or site directed mutagenesis reaction.

Features

For adhesive ends with 5 'or 3' protrusions, it can catalyze the generation of flat ends for subsequent flat end connections.

Specification

1. **Molecular weight:** approximately 68kDa (monomer).
2. **Activity definition:** The amount of enzyme required to catalyze the incorporation of 10nmol deoxyribonucleotides (dNTPs) into polynucleotides at 37℃ for 30 minutes is defined as one activity unit.
3. **Enzyme activity detection conditions:** 67mM potassium phosphate (pH7.4), 6.7mM $MgCl_2$, 1mM 2-mercaptoethanol, 0.033mM dATP, 0.033mM dTTP, 0.4MBq/mL [3H]-dTTP, 62.5μg/mL poly (dA dT) • poly (dA dT).
4. **Purity:** No DNA endonuclease, no RNase.
5. **Enzyme storage solution:** 25mM Tris HCl (pH 7.5), 0.1mM EDTA, 1mM DTT, 50% (v/v) glycerol.
6. **Reaction Buffer (10X):** 500mM Tris HCl (pH 8.0 at 25 ° C), 50mM MgCl2, 10mM DTT.
7. **Buffer compatibility:** The activity is 100% in the endonuclease reaction buffer solutions 1X O, 1X R, 1X Y, and 2X Y, and 100% in 1X B and 1X G; The activity was 100% in Taq, Pfu DNA polymerase, and M-MuLV reaction buffer.
8. **Inactivation or inhibition:** Heating at 75ºC for 10 minutes or adding an appropriate amount of EDTA can cause Klenow Fragment inactivation. Metal ion chelating agents, inorganic pyrophosphate (PPi), and high-dose inorganic phosphate (Pi) all have inhibitory effects on Klenow Fragment.

Protocol

1. **Flattening of the 5 'overhang end of double stranded DNA:**

1.1. Refer to the table below to set up the reaction system:

Digested DNA	10~15μL(0.1~4μg)
Reaction Buffer (10X)	2μL

dNTP Mixture (2.5mM each)	0.4μL
Klenow Fragment	0.5~2μL (1~4U)
Supplement deionized water without nucleases	To 20μL

1.2. After setting up the reaction system according to the table above, gently mix it (you can use a pipette to blow and mix or use a Vortex to mix at the lowest speed), and then centrifuge to precipitate the liquid.

1.3. Incubate at 37℃ for 10 minutes.

1.4. Incubate at 75℃ for 10 minutes to terminate the reaction.

2. Labeling of the 5 'overhanging end of double stranded DNA:

2.1. Refer to the table below to set up the reaction system:

Digested DNA	10~15μL(0.1~4μg)
Reaction Buffer (10X)	2μL
[α-^{32}P]-dNTP, ~15-30 TBq/mmol(400-800Ci/mmol)	0.74 MBq(20μCi)
or [α -^{32}P] - dNTP,~110 TBq/mmol (3000Ci/mmol)	2.96 MBq(80μCi)
3 dNTP Mixture (2.5mM each , without the labeled dNTP)	2μL
Klenow Fragment	0.5μL (1U)
Supplement deionized water without nucleases	To 20μL

2.2. After preparing according to the above system, gently mix (you can use a pipette to blow and mix or use a Vortex to gently mix at the lowest speed), and then centrifuge to precipitate the liquid.

2.3. Incubate at 30℃ for 15 minutes.

2.4. Incubate at 75℃ for 10 minutes to terminate the reaction

3. Other uses can refer to the above uses or appropriate literature materials.

Note

1. Enzymes should be stored in an ice box or on an ice bath during use, and immediately stored at -20ºC after use.
2. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
3. For your safety and health, please wear lab coats and disposable gloves when operating.