Klenow Fragment

Product description

Klenow Fragment is a proteolytic fragment derived from *Escherichia coli* DNA polymerase I. It retains the DNA polymerase activity and the 3'→5' exonuclease activity of the original enzyme, but lacks the 5'→3' exonuclease activity. Klenow Fragment is commonly used for DNA labeling by random priming, incorporating labeled nucleotides to generate DNA probes. It can also catalyze the formation of blunt ends from DNA fragments with 5' or 3' overhangs. Additionally, Klenow Fragment is widely employed in synthesizing the second strand of cDNA, facilitating the construction of double-stranded cDNA libraries. Due to the absence of 5'→3' exonuclease activity, Klenow Fragment does not degrade template DNA during DNA synthesis, thus providing greater stability and accuracy in experimental procedures.

Composition and storage conditions

Components \ Size	200 U	1000 U	Storage
Klenow Fragment (5 U/μL)	40 μL	200 μL	-20°C
Klenow Fragment Reaction Buffer (10×)	1.25 mL	1.25 mL	-20°C
Shipping: Dry Ice	**Shelf life:** 12 months		

Experimental operation

I Fill in 5' overhang ends or remove 3' overhang ends of dsDNA:

1) Thaw the components on ice, mix thoroughly, centrifuge, and place on ice for later use.

2) Set up the reaction system for DNA with 5 'or 3' overhang ends on ice according to the following table:

Reagent	Fill-in of 5' overhang ends	Removal of 3' overhang ends	Final Concentration
Nuclease-free Water	To 20 μL	To 20 μL	
dsDNA with 5’ Overhang	X μL	/	~0.5μM or 5-200ng/μL
dsDNA with 3’ Overhang	/	X μL	~0.5μM or 5-200ng/μL

Klenow Fragment Reaction Buffer (10×)	2 μL	2 μL	1×
dNTP Mix (2.5mM each)	0.4 μL	/	50 μM
Klenow Fragment (5 U/μL)	1 μL	1 μL	0.25 U/μL
Total Reaction Volume	20 μL	20 μL	

***Note:**

a. If many reactions are performed simultaneously, all solutions and enzymes listed in the table, except for the dsDNA with 5' overhang/dsDNA with 3'overhang, can be pre-mixed and aliquoted into individual reaction tubes. Finally, add the dsDNA with 5' overhang/dsDNA with 3' overhang.

b. If the dsDNA with 5' overhang/dsDNA with 3' overhang is an oligonucleotide, the final concentration can be approximately 0.5 μM. If it is digested plasmid DNA or similar, the final concentration can be approximately 5-200 ng/μL.

3) Invert, or gently pipette or gently vortex to thoroughly mix the reaction solution, and briefly centrifuge to collect the liquid to the bottom of the tube.

4) Incubate at 37 °C for 10 min.

5) Heat at 75 °C for 10 min to terminate the reaction.

II For other uses, you can search and refer to other appropriate literature and material.

Notes

1. Enzymes should be kept on ice during use and returned immediately to -20 ºC for storage after use.

2. This product is for scientific use only!

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com