

Klenow Fragment (3'-5' exo-)

Product Number: KL02

Shipping and Storage

-20°C

Components

| Component | KL02 | KL02 |
|--------------------------------------|--------|--------|
| | 200U | 1000U |
| Klenow Fragment (3'-5' exo-) (5U/μl) | 40 μl | 200 μl |
| 10×Klenow Buffer | 400 μl | 1.8 ml |

Description

Klenow Fragment (3'-5' exo -) is a mutated enzyme of DNA Klenow Fragment. This enzyme catalyzes the synthesis of complementary DNA to the template along the 3'-5' direction using dNTP as a substrate in the presence of templates and primers. Through point mutation modification, this enzyme lost both the activity of the 3'-5' exonuclease and the cleavage translation activity, while the point mutation modification made the 5'-3' polymerase activity of this enzyme stronger. This product is a recombinant enzyme expressed in Escherichia coli, with a molecular weight of approximately 68.1 kDa. Enzymes have high specific activity, good stability, and strong compatibility with other enzymes.

Unit definition

The amount of enzyme required to add 10nmol dNTP to acid insoluble substances within 30 minutes at 37°C is defined as 1 active unit (U).

Quality control

After multiple column purifications, SDS-PAGE detected a purity of over 99%; After testing, there was no contamination of nucleic acid endonucleases, exonucleases, phosphatases, and RNA enzyme activities.

Protocol

DNA terminal phosphorus plus T:

1. Refer to the following table to set up the reaction system

| Reagent | 25 μl reaction system |
|--------------------------------------|-----------------------|
| DNA to be added with T | 200 ng |
| 10×Klenow Buffer | 2.5 μl |
| dTTP (10μM) | 1 μl |
| Klenow Fragment (3'-5' exo-) (5U/μl) | 1.5 μl |
| ddH ₂ O | up to 25 μl |

2. After setting the reaction system according to the above table, gently mix and centrifuge to precipitate the liquid.
3. Incubate at 37°C for 30 minutes.
4. Purify the product fragment using a purification kit.

Note

1. Klenow Fragment (3'-5' exo -) cannot form a flat end because it lacks the activity of the 3'-5' exonuclease.
2. Enzymes should be stored in an ice box or ice bath when in use, and should be immediately stored at -20°C after use.