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KpnI

Cat # RE079
Pack Size: 500U
Volume: 50 µL
Storage: -20°C

Recognition Sequence: 5' GGTAC|C 3'
3' C|CATGG 5'

Kit content:

S.No	Component	RE079
1	KpnI	500U/50µL(10U/µL)
2	10X Universal Buffer	500 µL

Introduction

KpnI is a restriction endonuclease enzyme that recognizes and cleaves DNA at the specific palindromic hexanucleotide sequence 5'-GGTACC-3'. This type II restriction enzyme is isolated from the bacterium *Klebsiella pneumoniae*, from which its name is derived. The enzyme has been extensively utilized in molecular biology research, particularly in cloning applications where precise control over DNA manipulation is required. KpnI produces a "sticky" end cleavage, generating a four-base 3' overhang. This overhang can be valuable in constructing recombinant DNA by facilitating the joining of complementary DNA fragments.

Features

- Assayed on λ DNA
- Ligation/recutting assay: After 20-fold overdigestion with enzyme, >90% of the DNA fragments can be ligated and recut.
- Overdigestion assay: No nonspecific activity detected after incubation of 1 µg of λ DNA with 20 units of KpnI for 16 hours
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Protocol:

- The enzyme should not exceed 10 % of total reaction volume.
- Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
- Incubate 60 min. at 37 °C.

S.No	Component	50 µl Reaction
1	DNA	1 µg
2	10X Universal Buffer	5 µL
3	KpnI	2-5 units
4	Nuclease-free Water	to 50µL

Heat Inactivation:

Stop reaction by alternatively:

- Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM
- Heat Inactivation (20 min. at 65 °C)
- Spin Column DNA Purification (e.g. Cat.-No. PUR13-50)
- Gel Electrophoresis and Single Band Excision (e.g. Cat.-No. PUR12-50)
- Phenol-Chloroform Extraction or Ethanol Precipitation.

Supplied in : 10mM Tris-Hcl (pH 7.6), 50mM NaCl, 0.1mM EDTA, 1mM DTT, 100µg/ml BSA, 50% Glycerol.

Unit definition : One unit is defined as the amount of restriction enzyme required to completely digest 1 µg of lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µL.

Quality Control Assays

- Ligation of DNA fragments: DNA fragments are produced by an excessive over digestion of substrate DNA with each restriction endonuclease. These fragments are then ligated with T4 DNA Ligase at a 5' termini concentration of 0.1-1.0 µM. The ligated fragments are then recut with the same restriction endonuclease. Ligation can only occur if the 3' and 5' termini are left intact, and only those molecules with a perfectly restored recognition site can be recleaved. A normal banding pattern after cleavage indicates that both the 3' and 5' termini are intact, and the enzyme preparation is free of detectable exonucleases and phosphatases.
- DNA digestion with restriction enzymes may be affected by some types of methylation.
- In general, it is recommended to use 5–10 units of enzyme per µg of plasmid DNA, and 10–20 units for genomic DNA in a 1-hour digest. Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity.
- All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5' exonuclease/ 5' phosphatase, as well as nonspecific single- and doublestranded DNase activities.