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SpeI

Cat # RE173
Pack Size: 200U
Volume: 20 µL
Storage: -20°C

Recognition Sequence: 5' A↓CTAGT 3'
3' TGATC↑A 5'

Kit content:

S.No	Component	RE173
1	SpeI	200U/20µL(10U/µL)
2	10X Universal Buffer	200 µL

Introduction

SpeI Restriction Enzyme is a high-quality enzyme derived from the bacterium Sphingomonas species. It belongs to the Type II restriction enzyme family and is widely used in molecular biology applications for DNA manipulation and cloning purposes. SpeI specifically recognizes the DNA sequence 5'-ACTAGT-3' and cleaves it, generating cohesive or sticky ends. Its is sourced from an *E.coli* strain, that carries the cloned SpeI gene from Sphingomonas.

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 SpeI for 16 hours.

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	SpeI	2-5 units
4	Nuclease-free Water	to 50µL

Enzyme 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.