FlyCut™ SpeI

Cat.No. JS601

Storage: at -20°C for two years

Concentration: 10,000 units/ml

Recognition Site

5'…A▼CTAGT…3'

3'…TGATC▲A…5'

Description

FlyCut™ SpeI is expressed and purified from *E.coli* that carries the recombinant SpeI gene. The molecular weight is 21.7 kDa, with the recognition site at A^CTAGT. The reaction is conducted for 5-10 minutes at 37°C, and heat-inactivated at 80°C for 20 minutes. This enzyme is not sensitive to dam, dcm or mammalian CpG methylation.

Enzyme Properties

Fast digestion in 5-10 minutes with high fidelity

Application

Genomic DNA, plasmid DNA, PCR product

Kit Contents

Component	JS601-01	JS601-02
FlyCut™ SpeI	250 units	500 units
10×*FlyCut*™ Buffer	1 ml	1 ml
10×DNA Loading Buffer	1 ml	1 ml

Unit Definition

One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Quality Control

Ligation and re-cutting: After 10-fold overdigestion with *FlyCut*™ SpeI, more than 95% of the DNA fragments can be ligated with T4 DNA ligase at 25°C. Of these ligated fragments, more than 95% can be recut.

16-Hour incubation: A 50 μl reaction containing 1 μg of DNA and 10 units of enzyme incubated for 16 hours results in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Blue/White screening (Terminal integrity): A DNA vector is digested at a unique site within lacZα gene with a 10-fold excess of enzyme, and then ligated, transformed and plated on X-gal/IPTG plate. Successful expression of the β-galactosidase indicates that lacZα gene remains integrity after cloning. A blue colony represents an intact gene, and a white colony represents an interrupted gene. To be Blue/White certified, enzymes must produce fewer than 3% white colonies.

Exonuclease activity: After incubation for 4 hours at 37°C, a 50 μl reaction containing 100 units of enzyme and 1 μg ^{3}H DNA releases less than 0.1% radioactive substance.

Endonuclease activity: After incubation for 4 hours at 37°C, a 50 μl reaction containing 15 units of enzyme with 1 μg pBR322 RFI DNA results in less than 10% conversion from RFI to RFII.

Storage Buffer

20 mM Tris-HCl pH7.4, 250 mM NaCl, 0.1 mM EDTA, 1.5 mM DTT, 400 μg/ml BSA, 50% Glycerol

10×*FlyCut*™ Buffer

500 mM Tris-Ac pH7.9, 1 M KAc, 120 mM $MgAc_2$, 1 mg/ml BSA

Reaction Components

Component	20 μl Volume	50 μl Volume
DNA	≤1 μg	≤2 μg
10×*FlyCut*™ Buffer	2 μl	5 μl
FlyCut™ SpeI	0.5 μl	1 μl
ddH_2O	to 20 μl	to 50 μl

Prior to use, please completely mix the *FlyCut*™ Buffer. Increase the volume of enzyme, in case of digestion of >2 μg DNA or incomplete digestion, but the total volume of enzyme should be less than 1/10 of the reaction system.

Incubation for 5-10 minutes at 37°C. Enzyme is inactivated by adding 10×DNA Loading Buffer to a final concentration at 1×, or by heating at 80°C for 20 minutes.

Notes

• Thaw the 10×*FlyCut*™ Buffer completely and mix well before use.

• Low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity.

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