FlyCut® XbaI

Cat.No. JX101
Storage: at -20°C for two years
Concentration: 20,000 units/ml

Recognition Site
5'…T▼CTAGA…3'
3'…AGATCT▲…5'

Description

FlyCut® XbaI is expressed and purified from *E.coli* that carries the recombinant XbaI gene. The molecular weight is 24.7 kDa, with the recognition site at T^CTAGA. The reaction is conducted for 5-15 minutes at 37°C, and heat-inactivated at 65°C for 20 minutes. This enzyme is not sensitive to dcm or mammalian CpG methylation, but sensitive to dam methylation.

Enzyme Properties

Fast digestion in 5-15 minutes with high fidelity

Application

Genomic DNA, plasmid DNA, PCR product

Kit Contents

Component	JX101-01	JX101-02
FlyCut® XbaI	1,500 units	2×1,500 units
10×*FlyCut*® Buffer	500 µl	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*® XbaI, 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	Volume	Volume	Final Concentration
DNA	≤1 µg	1-2 µg	as required
10×*FlyCut*® Buffer	2 µl	5 µl	1×
FlyCut® XbaI	0.5 µl	1 µl	-
Nuclease-free Water	Variable	Variable	-
Total volume	20 µl	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 5-15 minutes at 37°C. Enzyme is inactivated by adding 10×DNA Loading Buffer to a final concentration at 1×, or by heating at 65°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.