AllBio Not I-Fast Cut

Cat. No. ABTGREN401
Storage： at -20°C for two years
Concentration： 20,000 units/ml

Recognition Site
5'...GC▼GGCCGC...3'
3'...CGCCGG▲CG...5'

Description

AllBio Not I-Fast Cut is expressed and purified from E.coli that carries the recombinant Not I gene. The molecular weight is 43.3 kDa, with the recognition site at GC^GGCCGC. 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 dam or dcm methylation, but sensitive to mammalian CpG methylation.

Enzyme Properties

Fast digestion in 5-15 minutes with high fidelity

Application

Genomic DNA, plasmid DNA, PCR product

Kit Contents

Component	ABTGREN401-01	ABTGREN401-02
AllBio Not I-Fast Cut	250 units	2×250 units
10×FastCut Buffer	250 ul	250 ul
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 AllBio Not I-Fast Cut, 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×FastCut Buffer

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

AllBio NotI-Fast Cut

Reaction Components

Component	20 µl Volume	50 µl Volume
DNA	≤1 µg	1-2 µg
10×FastCut Buffer	2 µl	5 µl
AllBio Not I-Fast Cut	0.5 µl	1 µl
ddH_2O	to 20 µl	to 50 µl

For digestion of >2 µg DNA , please adjust the amount of each component based on above table.

Reaction Condition

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×FastCut 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.