FlyCut® NotI

Cat.No. JN401

Storage: at -18℃ or below for two years

Concentration: 20,000 units/ml

Recognition Site

5'…GC▼GGCCGC…3'

3'…CGCCGG▲CG…5'

Description

FlyCut® NotI is expressed and purified from *E.coli* that carries the recombinant NotI gene. The molecular weight is 43.3 kDa, with the recognition site at GC^GGCCGC. The reaction is conducted 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 minutes

• Universal buffer

• Star activity-free

Application

Genomic DNA, plasmid DNA, PCR product

Kit Contents

Component	JN401-01	JN401-02
FlyCut® NotI	250 units	2×250 units
10×*FlyCut*® Buffer	250 µl	250 µl
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*® NotI, 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

Recommended single digestion reaction system

Component	Volume	Volume
DNA	≤1 μg	1-2 μg
10×*FlyCut*® Buffer	2 μl	5 μl
FlyCut® NotI	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.

Different DNA will have different effects of enzyme digestion due to their different structures, so the reaction time can be adjusted according to the digestion effect.

Reaction conditions

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.

Recommended double digestion reaction system

Component	Volume
DNA	≤2 μg
10×*FlyCut*® Buffer	5 μl
FlyCut® Enzyme I	1 μl
FlyCut® Enzyme II	1 μl
Nuclease-free Water	Variable
Total volume	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.

Different DNA will have different effects of enzyme digestion due to their different structures, so the reaction time can be adjusted according to the digestion effect.

Reaction conditions

Incubation for 5-15 minutes at the recommended reaction temperature. Enzyme is inactivated by adding 10×DNA Loading Buffer to a final concentration at 1×, or by heating.

If the two enzymes require different reaction temperatures, please refer to the “Notes for Double Digestion”.

Notes for Double Digestion

The FlyCut Buffer ensures 100% activity for any two enzymes while minimizing star activity.

- Follow the recommended conditions for setting up the reaction system: The glycerol concentration in the reaction should be <5% to avoid star activity. For example, in a 50 μl reaction system, the total enzyme volume should not exceed 5 μl.
- Incubate at the recommended temperature and time, overnight incubation is not advised for double digestion.
- If the two enzymes require different reaction temperatures: Add the first enzyme and incubate at its recommended temperature (e.g., SmaI at 25°C), heat-inactivate the first enzyme before adding the second enzyme, then incubate at its optimal temperature.

For research use only, not for clinical diagnosis.

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