

## AllBio Nde I-Fast Cut



Cat. No. ABTGREN201

Storage : at -20°C for two years

Concentration : 20,000 units/ml

Recognition Site

5'...CA<sup>▼</sup>TATG...3'

3'...GTATAC<sup>▲</sup>...5'

### Description

AllBio Nde I-Fast Cut is expressed and purified from *E.coli* that carries the recombinant Nde I gene. The molecular weight is 46.5 kDa, with the recognition site at CA<sup>▼</sup>TATG. The reaction is conducted for 5-10 minutes at 37°C, and heat-inactivated at 65°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	ABTGREN201-01	ABTGREN201-02
AllBio Nde I-Fast Cut	2,000 units	4,000 units
10×FastCut 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 AllBio Nde I, 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 <sup>3</sup>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<sub>2</sub>, 1 mg/ml BSA

## AllBio Nde I-Fast Cut



### Reaction Components

Component	20 $\mu$ l Volume	50 $\mu$ l Volume
DNA	$\leq 1 \mu\text{g}$	$\leq 2 \mu\text{g}$
10 $\times$ FastCut Buffer	2 $\mu$ l	5 $\mu$ l
AllBio Nde I-Fast Cut	0.5 $\mu$ l	1 $\mu$ l
ddH <sub>2</sub> O	to 20 $\mu$ l	to 50 $\mu$ l

Prior to use, please completely mix the FastCut Buffer. Increase the volume of enzyme, in case of digestion of  $>2 \mu\text{g}$  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 $\times$ DNA Loading Buffer to a final concentration at 1 $\times$ , or by heating at 65°C for 20 minutes.

### Notes

- Thaw the 10 $\times$ 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.