

NcoI

Cat # RE047

Pack Size: 500U/50uL(10U/u)

Storage: -20°C

Recognition Sequence: 5' C^CATGG 3'
3' GGTAC^C 5'

Optimal Buffer: 10x Universal Buffer □ 1mL



Introduction

NcoI is a restriction endonuclease derived from an *E. coli* strain with a cloned NcoI gene from *Neisseria corrodens*. Recognizing and cleaving the DNA sequence C[^]CATGG, NcoI functions optimally at 37°C. It's known for its precision, stability, and resistance to dam-methylation, making it a vital tool in DNA manipulation and genetic engineering. Heat-inactivation at 80°C provides a simple way to stop its enzymatic activity.

Features

- Assayed on λ DNA
- Heat inactivation: 65°C for 20 minutes
- Ligation/recutting assay: After 20-fold overdigestion with NcoI, >90% of the DNA fragments can be ligated and recut
- Overdigestion assay: No nonspecific activity detected after incubation of 1 µg of λ DNA with 20 units of NcoI for 16 hours

Protocol

Component	50 µl Reaction
DNA	1 µg
10x Universal Buffer	5 µl
NcoI	2-5 units
Nuclease-free Water	to 50µl

- Reaction setup (This is just an example to show the relative concentrations and volumes in the reaction may wish to set up a reaction ranging from 10 µl to 200 µl or more
- Incubate at 37°C for 15 minutes. Longer incubation times (sometimes overnight) may be followed as per specific requirement
- Heat inactivate enzyme at 65°C for 10 mins.
- Please note that supercoiled plasmid DNA and PCR fragments may have varied rate of cleavage and sometime needs more time to completely digest

Certificate of Analysis

Source	: An <i>E. coli</i> strain, that carries the cloned NcoI gene from <i>Neisseria corrodens</i>
Supplied in	: 10mM Tris-Hcl (pH 7.6), 50mM NaCl, 0.1mM EDTA, 1mM DTT, 100ug/ml, BSA, 50% Glycerol.
Reaction Conditions	: 1x Universal Buffer, Incubate at 37°C for 10 min
Unit definition	: One unit of NcoI is defined as the amount of enzyme required to completely digest 1 µg of lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.
Optimal temperature	: 37°C
Heat Inactivation	: Enzyme is inactivated by incubation at 65°C for 10 minutes.

Quality Control Assays

Ligation of DNA fragments

DNA fragments are produced by an excessive over digestion of substrate DNA with each restriction endonuclease. These fragments are then ligated with T4 DNA Ligase at a 5' termini concentration of 0.1-1.0 µM. The ligated fragments are then recut with the same restriction endonuclease. Ligation can only occur if the 3' and 5' termini are left intact, and only those molecules with a perfectly restored recognition site can be recleaved. A normal banding pattern after cleavage indicates that both the 3' and 5' termini are intact, and the enzyme preparation is free of detectable exonucleases and phosphatases.

DNA digestion with NcoI may be affected by some types of methylation

* In general, it is recommended to use 5–10 units of enzyme per µg of plasmid DNA, and 10–20 units for genomic DNA in a 1-hour digest. Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity.

Reagents Supplied with Enzyme: 10x Universal Buffer.