

## Not I

Cat #  RE203S,  RE203L

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

2500U/250uL(10U/uL)

Storage: -20°C

Recognition Sequence: 5' GC|GGCCGC 3'  
3' CGCCGG|CG 5'

Optimal Buffer: 10x Universal Buffer  1mL  2mL



### Introduction

NotI is a widely used Type II restriction enzyme that recognizes and cleaves DNA at a specific recognition sequence. The recognition sequence for NotI is 5'-GCGGCCGC-3'. One of the key features of NotI is its ability to generate cohesive or sticky ends upon DNA digestion. The cleavage of DNA by NotI leaves 5' overhangs with a sequence of 5'-GCGGCCGC-3' on one strand and its complementary sequence on the other strand. These sticky ends can then be easily ligated with other DNA fragments that have complementary overhangs generated by the same enzyme or compatible enzymes. The cohesive end property of NotI allows for efficient and precise DNA cloning.

### Features

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

### Protocol

Component	50 $\mu$ l Reaction
DNA	1 $\mu$ g
Universal Buffer	5 $\mu$ l
Not I	2-5 units
Nuclease-free Water	to 50 $\mu$ l

- 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  $\mu$ l to 200  $\mu$ l 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 NotI gene from <i>Nocardia otitidis-caviarum</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 NotI is defined as the amount of enzyme required to completely digest 1 $\mu$ g of lambda DNA in 1 hour at 37°C in a total reaction volume of 50 $\mu$ l.
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  $\mu$ M. 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 NotI** may be affected by some types of methylation

\* In general, it is recommended to use 5–10 units of enzyme per  $\mu$ g 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