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NheI

Cat # ☐ **RE063**
Pack Size: ☐ **500U/50uL(10U/ul)**
Storage: -20°C

Recognition Sequence: 5' G↓CTAGC 3'
3' CGATC↑G 5'

Optimal Buffer: **10x Universal Buffer** ☐ **1mL**

Introduction

NheI is a Type II restriction enzyme that recognizes and cleaves DNA at the palindromic sequence GCTAGC, generating sticky ends. This enzyme was first isolated from the bacterium *Neisseria mucosa heidel*bergensis, hence its name. NheI is commonly used in molecular biology for DNA cloning, restriction mapping, and other applications that require precise DNA fragment cutting. The enzyme is known for its high specificity, efficiency, and stability, making it a popular choice for many molecular biology experiments.

Features

- Assayed on λ DNA
- Heat inactivation: 65°C for 20 minutes
- Ligation/recutting assay: After 20-fold overdigestion with Xba I, >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 Xba I for 16 hours

Protocol

- 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

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

- Incubate at 37°C for 10 minutes. Longer incubation times (sometimes overnight) may be followed as per digestion efficiency
- 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 *E.coli* strain, that carries the cloned NheI gene from *Neisseria mucosa heidelbergensis*
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 NheI 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 Nhe I 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 .