

CERTIFICATE OF ANALYSIS

# NheI

**#ER0972**      2500 u

**Lot:**                      **Expiry Date:**

5'...**G↓C T A G C**...3'

3'...**C G A T C↑G**...5'

Concentration:      10 u/μl  
Source:                *Neisseria mucosa heidelbergensis*  
Supplied with:      1 ml of 10X Buffer Tango™

**Store at -20°C**



In total 2 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer Tango™** (for 100% NheI digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate,  
66 mM potassium acetate, 0.1 mg/ml BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of NheI required to digest 1 μg of lambda DNA-HindIII fragments in 1 hour at 37°C in 50 μl of recommended reaction buffer.

**Dilution**

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

**Double Digests**

Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas Restriction Enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to [www.fermentas.com/doubledigest](http://www.fermentas.com/doubledigest) to choose the best buffer for your experiments.

**Storage Buffer**

NheI is supplied in: 10 mM Tris-HCl (pH 8.0 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
DNA (0.5-1 $\mu$ g/ $\mu$ l)	1 $\mu$ l
NheI	0.5-2 $\mu$ l*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

The digestion reaction may be scaled either up or down.

## Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 $\mu$ l (~0.1-0.5 $\mu$ g of DNA)
nuclease-free water	18 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
NheI	1-2 $\mu$ l*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Note.

## Thermal Inactivation

NheI is inactivated by incubation at 65°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

B	G	O	R	Tango™	2X Tango™
100	20-50	0-20	0-20	100	0-20

### Methylation Effects on Digestion

Dam: never overlaps – no effect.  
Dcm: never overlaps – no effect.  
CpG: may overlap – cleavage impaired.  
EcoKI: never overlaps – no effect.  
EcoBI: never overlaps – no effect.

### Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1  $\mu$ g of lambda DNA in 16 hours at 37°C.

### Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1  $\mu$ g of agarose-embedded lambda DNA in 16 hours.

### Compatible Ends

XmaJI, BcuI, Eco130I, XbaI

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
1	0	1	0	0	0	0

### Note

A large excess of NheI (7.5 u/ $\mu$ g DNA x 16 hours) may result in star activity.

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with NheI (5 u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with NheI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.05 μM. More than 95% of these sites can be recut.

## Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of NheI for 4 hours.

## Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 5 units of NheI for 16 hours. After religation and transformation, the background level of white colonies was 0.1%.

Quality authorized by:



Jurgita Zilinskiene

## PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to [www.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.