

## PRODUCT INFORMATION

# NheI

#ER0971      500 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



BSA included

[www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio)

## RECOMMENDATIONS

**1X Thermo Scientific Tango Buffer** (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 provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to [www.thermoscientific.com/doubledigest](http://www.thermoscientific.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 µL  
10X Buffer Tango 2 µL  
DNA (0.5-1 µg/µL) 1 µL  
NheI 0.5-2 µL\*
- 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 µL (~0.1-0.5 µg of DNA)  
nuclease-free water 18 µL  
10X Buffer Tango 2 µL  
NheI 1-2 µL\*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Overdigestion Assay.

## Thermal Inactivation

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

## ENZYME PROPERTIES

### Enzyme Activity in Thermo Scientific 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 µg 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 µg of agarose-embedded lambda DNA in 16 hours.

### Compatible Ends

BcuI, Eco130I, XbaI, XmaJI

### Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|---|-------|--------|-------|----------|----------|------------|
| 1 | 0     | 1      | 0     | 0        | 0        | 0          |

### Note

- Supercoiled plasmids may require up to 10-fold more NheI for complete digestion than linear DNAs (e.g. 10 units are required to cleave 1 µg of pBR322 DNA).

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# CERTIFICATE OF ANALYSIS

## 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 and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

## 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.

Quality authorized by:



Jurgita Zilinskiene

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