

## PRODUCT INFORMATION

# NcoI

#ER0575 1000 U

Lot: \_\_\_\_\_ Expiry Date: \_\_\_\_\_

5'...C↓C A T G G...3'

3'...G G T A C↑C...5'

Concentration: 10 U/μL

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% NcoI 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 NcoI required to digest 1 μg of lambda DNA 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

NcoI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Rev.10

## 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
NcoI	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
NcoI	1-2 $\mu$ L*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Overdigestion Assay.

## Thermal Inactivation

NcoI 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
20-50	20-50	20-50	50-100	100	100

### Methylation Effects on Digestion

Dam: never overlaps – no effect.  
Dcm: never overlaps – no effect.  
CpG: never overlaps – no effect.  
EcoKI: never overlaps – no effect.  
EcoBI: never overlaps – no effect.

### Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1  $\mu$ g of DNA in 16 hours at 37°C.

### Digestion of Agarose-embedded DNA

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

### Compatible Ends

AflIII, Psci, Btgi, Eco130I, FatI, PagI.

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
4	0	0	0	0	0	0

For **CERTIFICATE OF ANALYSIS** see back page

# CERTIFICATE OF ANALYSIS

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with NcoI (5 U/ $\mu$ g lambda DNA  $\times$  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 NcoI for 4 hours.

## Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

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.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio) for Material Safety Data Sheet of the product.

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