

PRODUCT INFORMATION

NmuCI (Tsp45I)

#ER1511 200 U

Lot: ___ Expiry Date: _

5'... **↓G T S A C** ...3' 3'... **C A S T G**↑...5'

Concentration: 10 U/µL

Source: Neisseria mucosa C9-2 Supplied with: 1 mL of 10X Buffer R

1 mL of 10X Buffer Tango

Store at -20°C











BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer R (for 100% NmuCl digestion) 10 mM Tris-HCl (pH 8.5), 10 mM MgCl₂, 100 mM KCl, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of NmuCl required to digest 1 μ g lambda DNA in 1 hour at 37°C in 50 μ L 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

Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango $^{\text{\tiny TM}}$ Buffer. Please refer to

<u>www.thermoscientific.com/doubledigest</u> to choose the best buffer for your experiments.

1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Storage Buffer

NmuCl is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 100 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~R $2~\mu L$ $DNA~(0.5-1~\mu g/\mu L)$ $1~\mu L$ NmuCl $0.5-2~\mu L$

- 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 μ L (~0.1-0.5 μ g of DNA) nuclease-free water 18 μ L 10X Buffer R 2 μ L NmuCl 1-2 μ L

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

В	G	0	R	Tango	2X Tango
0-20	20-50	50-100	100	20-50	50-100

Methylation Effects on Digestion

Dam: never overlaps – no effect. Dcm: never overlaps – no effect.

CpG: may overlap – cleavage impaired.

EcoKI: never overlaps – no effect.

EcoBl: may overlap – effect not determined.

Stability during Prolonged Incubation

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

Compatible Ends

Eco91I (G↓GTCACC) MaeIII (↓GTGAC)

Number of Recognition Sites in DNA

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
81	8	9	4	4	4	8

For **CERTIFICATE OF ANALYSIS** see back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with NmuCl (10 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of NmuCl for 4 hours.

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 for Material Safety Data Sheet of the product.

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