Thermo SCIENTIFIC

PRODUCT INFORMATION

NotI

#ER0591 300 u

Lot: **Expiry Date:**

5'...**G C↓G G C C G C**...3'
3'...**C G C C G G↑C G**...5'

Concentration: 10 u/µl
Supplied with: 1 ml of 10X Buffer O
1 ml of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/fermentas

RECOMMENDATIONS

1X Buffer O (for 100% NotI digestion)
50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/ml BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of NotI required to digest 1 µg pTZ19RJL2 DNA-BseLI 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
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™ Buffer. Please refer to to www.fermentas.com/doubledigest 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

NotI is supplied in: 20 mM Tris-HCl (pH 7.8 at 25°C), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.02% Triton X-100, 0.2 mg/ml BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer O	2 µl
DNA (0.5-1 µg/µl)	1 µl
NotI	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 O	2 µl
NotI	1-2 µl*

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

* This volume of the enzyme is recommended for preparations of standard concentrations (10 u/µl), whereas HC enzymes (50 u/µl) should be diluted with Dilution Buffer to obtain 10 u/µl concentration.

Thermal Inactivation

NotI is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked.
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 µg of Ad2 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 Adenovirus-2 DNA in 16 hours.

Compatible Ends

Bsp120I, CfrI, Eco52I

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19	Ad2
0	0	0	0	0	0	0	7

Note

Supercoiled plasmids may require up to 5-fold more NotI for complete digestion than linear DNAs.

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 NotI (10 u/µg pTZ19RJL2 DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with NotI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.5 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 NotI for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of NotI for 16 hours. After religation and transformation, the background level of white colonies was <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.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.