Thermo SCIENTIFIC

PRODUCT INFORMATION

Nb.Bpu10I

#ER1681 1000 U

Lot: ___ **Expiry Date: _**

5'...**C C T N A G C**...3'
3'...**G G A N T↑C G**...5'

Concentration: 5 U/µL
Source: *E.coli* that carries the wild type *bpu10IRα* and the mutagenized *bpu10IRβ* genes from *Bacillus pumilus* RFL10
Supplied with: 1 mL of 10X Buffer R
1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

DESCRIPTION

Nb.Bpu10I is a site and strand specific endonuclease artificially engineered from R.Bpu10I that cleaves only one strand of the DNA within its recognition sequence on double stranded DNA substrate.

RECOMMENDATIONS

1X Buffer R (for 100% Nb.Bpu10I digestion)
10 mM Tris -HCl (pH 8.5), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of Nb.Bpu10I required to convert 1 µg of supercoiled pBR322 DNA to circular form 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.

Storage Buffer
Nb.Bpu10I is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 200 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Thermal Inactivation
Nb.Bpu10I 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	20-50	20-50	**100**	20-50	50-100

Stability during Prolonged Incubation

A minimum of 0.3 units of the enzyme is required for complete conversion of 1 µg of pBR322 DNA in 16 hours at 37°C into circular form.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
19	7	1	0	0	0	4

Note

Nb.Bpu10I may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

APPLICATIONS

- Production of single-stranded circular DNA from supercoiled double-stranded plasmids *in vitro* with subsequent use in DNA sequencing, site-specific mutagenesis, etc.
- Creation of nested deletions.
- Vector preparation for ligation independent cloning method.
- Preparations of covalently closed, double-stranded linear DNA molecules.

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Nonspecific Nicking Assay

Incubation of 10 units of Nb.Bpu10I with 1 µg pUC19 DNA (lacking the recognition sequence of Bpu10I) for 1 hour at 37°C in 50 µL reaction buffer resulted in 8.5% conversion to circular form.

Double Strand Endonuclease Assay

Incubation of 1 unit of Nb.Bpu10I with 1 µg pBR322 DNA for 1 hour at 37°C in 50 µL reaction buffer resulted in 3.5% conversion to linear form.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Nb.Bpu10I 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 for Material Safety Data Sheet of the product.