

Nt.BstNBI

REF: YS0203

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



Storage Condition

-20°C

Components

Components	Amount
Nt.BstNBI (10 U/μl)	200 μl
10× Cut Buffer C	2×1 ml

Description

Nt.BstNBI is a nicking endonuclease that only cuts one strand of the dsDNA substrate, resulting in nicks on the dsDNA without complete cleavage.

Recommended Reaction Conditions

1× Cut Buffer C;
Incubate at 55°C ;
Refer to "Protocol for DNA Digestion" for reaction setup.
This product has 100% activity when performing enzymatic digestion reactions at 37°C .
This product has 25% activity in CutOne® reaction buffer.

Heat Inactivation

Incubation at 80°C for 20 minutes.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to completely convert 1 μg of supercoiled pUC19 DNA into the open-circular form in a 50 μl reaction system at 55°C for 1 hour.

Quality Control

Prolonged Incubation


A 10 μl reaction containing 10 U of Nt.BstNBI and the supercoiled pUC19 DNA substrate were incubated at 55°C for 16 hours, and no change in the open-circular DNA was detected by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 U of Nt.BstNBI incubated for 1 hours at 37°C results in >90% of the substrate RNA remains intact as determined by agarose.

Icon Descriptions

 The enzyme's optimum reaction temperature is 55°C .

 The enzyme can be heat inactivated at by incubation 80°C for 20 minutes.

Method of application

1. Protocol for DNA Digestion

① Combine the following reaction components on ice in the order indicated:

ddH ₂ O	up to 50 μl
10× Cut Buffer C	5 μl
DNA ^a	1 μg
Nt.BstNBI (10 U/μl)	1 μl
Total	50 μl

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected;

- ② Mix gently and spin down.
- ③ Incubate at 55°C for 30 min~1 h.
- ④ Optional: Inactivate the enzyme by heating at 80°C for 20 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.

2. Notice

- ① The volume of enzyme added to the reaction mixture should not exceed 10% of the total volume to avoid star activity caused by excessive glycerol in the enzyme storage buffer.
- ② The additives (e.g., glycerol, salt) in the enzyme storage buffer are the same as the contaminants in the substrate solution (e.g., salt, EDTA, or ethanol, etc.). Therefore, the smaller the reaction volume, the stronger the digestion inhibition effect.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
61	10	4	3	4	5	8	40

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	No effect	No effect	No effect