Nb.BsrDI

REF: EG23514S

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

65 80

Storage Condition

-20℃

Components

Components	Amount
Nb.BsrDI (10 U/μl)	200 μl
10× CutOne™ Buffer	2×1 ml

Description

Nb.BsrDI is a nicking endonuclease that cuts only one strand of the dsDNA substrate; it introduces a nick into the dsDNA substrate without cleaving the dsDNA.

Recommended Reaction Conditions

1× CutOne™ Buffer;
Incubate at 65℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.
This product has 50% activity when performing enzymatic digestion reactions at 37℃ .

Heat Inactivation

Incubation at 80℃ for 20 minutes.

Definition of Activity Unit

One unit of activity is defined as the amount of enzyme required to completely convert 1 μg of supercoiled pUC19 DNA into open circular form in a 50 μl reaction system within 1 hour at 50℃ .

Quality Control

Ligation and Recuting

A 10 μl reaction containing supercoiled pUC19 DNA substrate and 10 U of Nb.BsrDI incubated for 16 hours at 65℃ results in no detectable degradation of the open circular DNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 U of Nb.BsrDI incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Icon Descriptions

65 The enzyme's optimum reaction temperature is 65℃ .

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

Protocol

1. Protocol for DNA Digestion

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

ddH_2O	up to 50 μl
10× CutOne™ Buffer	5 μl
DNA[a]	1 μg
Nt.BsrDI (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 Incubation at 65℃ for 30 minutes~1 hour.
④ Optional: Inactivate the enzyme by heating at 80℃ 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
44	4	2	2	2	4	3	14

Methylation Effects on Digestion

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

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