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 EB 20 80

Storage Condition

-20℃

Components

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

Description

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

Nb.BsrDI shows superior activity in the universal CutOne® and CutOne® Color Buffer. CutOne® Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the CutOne® Color Buffer migrates with 2.5 kb double-strand DNA fragments in a 1% agarose gel, and the yellow dye migrates with 10 bp double-strand DNA fragments in a 1% agarose gel.

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 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 65℃ for 1 hour.

Quality Control

Prolonged Incubation

A 10 μl reaction containing 10 U of Nb.BsrDI and the supercoiled pUC19 DNA substrate were incubated at 65℃ 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 Nb.BsrDI incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Icon Descriptions

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

EB Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the EcoBI methylase.

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

Method of application

1. Protocol for DNA Digestion

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

ddH_2O	up to 50 μl
10× CutOne® Buffer or 10× CutOne® Color Buffer	5 μl
DNA[a]	1 μg
Nb.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 Incubate at 65℃ for 30 min~1 h.

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	Impaired

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com