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Nb.BsrDI

Product Number: RE05880

Shipping and Storage

-20℃.

Components

Component	Specifications
Nb.BsrDI (10 U/μL)	200μL
10× FlashCut™ Buffer	2×1mL
10× FlashCut™ Color Buffer	2×1mL

Description

Nb.BsrDI is a type of endonuclease that cleaves only one strand of dsDNA substrate; Create incisions on the dsDNA substrate without incising the dsDNA.

Suggested reaction conditions

1. 1 × FlashCut™ Buffer.
2. Incubate at 65℃.
3. Prepare the reaction system according to the "DNA rapid enzyme digestion process".
4. This product has 50% activity when subjected to enzymatic digestion at 37℃.

Inactivation conditions

Incubate at 80℃ for 20 minutes.

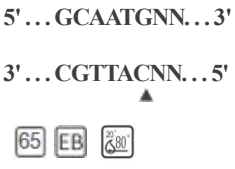
Definition of Activity

1 active unit (U) refers to the amount of enzyme required to completely convert 1μg of supercoiled pUC19 DNA into an open-loop form within 1 hour at 65℃ in a 50μL reaction system.

Quality control

1. Ultra long incubation test: incubate 10 U Nb. BsrDI and super spiral pUC19 DNA substrate at 65℃ for 16 h, and detect no change in open loop DNA by agarose gel electrophoresis.
2. RNase residue detection: incubate 10 U Nb. BsrDI and 500ng RNA at 37℃ for 1 h, and use agarose gel electrophoresis to detect that more than 90% of RNA remains intact.

Icon annotation



1. 65 The optimal reaction temperature is 65℃.
2. EB For DNA methylated by EcoBI, splicing may be hindered
3. 80 Inactivation condition: Incubate at 80℃ for 20 minutes.

Protocol

1. DNA rapid enzymatic digestion process

1.1. Prepare the reaction system on ice according to the recommended sample addition sequence as follows:

ddH_2O	up to 50μL
10×FlashCut™ Buffer or 10×FlashCut™ Color Buffer	5μL
Substrate DNA	1μg
Nb.BsrDI (10 U/μL)	1μL
Total	20μL

The DNA substrate should not contain phenol, chloroform, ethanol EDTA、 Detergent or high concentration salt, otherwise it will affect the activity of Nb.BsrDI enzyme;

1.2. Gently suck or tap the tube wall to mix well (without vortexing), then centrifuge instantly to collect hanging wall droplets;

1.3. Incubate at 65℃ for 30 minutes to 1 hour;

1.4. Incubate at 80℃ for 20 minutes to inactivate the enzyme, stop the reaction, or terminate the reaction through adsorption column or phenol/chloroform purification.

2. Note

2.1. The volume of enzyme added to the reaction system should not exceed 10% of the total volume to avoid excessive glycerol in the enzyme causing star activity;

2.2. The additives (such as glycerol, salt) in the storage buffer for restriction endonucleases are the same as the pollutants (such as salt, EDTA, or ethanol) in the substrate solution. The smaller the reaction volume, the stronger the inhibitory effect on the enzyme cleavage reaction.

The number of enzyme cleavage sites in different DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
44	4	2	2	2	4	3	14

The influence of methylation modification

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	no effect	no effect	Shear effect