

SspDI (KasI)

Product Number: RE0585

Shipping and Storage

-20°C.

Components

Component	Specifications
SspDI (KasI)	25μL
10× FlashCut™ Buffer	1mL
10× FlashCut™ Color Buffer	1mL

Description

SspDI belongs to the conventional restriction enzyme series and can recognize G[^]GCGCC sequences. Unlike the Thunder series of rapid endonucleases, SspDI requires a longer time for enzyme cleavage to achieve complete cleavage of the DNA substrate, but this enzyme still uses the Thunder restriction enzyme universal reaction buffer FlashCut™ Buffer, Can achieve dual enzyme digestion.

Suggested reaction conditions

- 1 × FlashCut™ Buffer solution.
- Incubate at 37°C.
- Prepare the reaction system according to the "DNA rapid enzyme digestion process".

Inactivation conditions

Incubate at 80°C for 20 minutes.

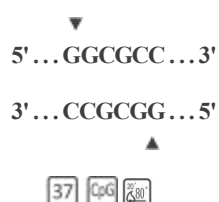
Definition of Activity

1 active unit (U) refers to the reaction in a 50μL reaction system, The amount of enzyme required to completely cleave 1μg pBR322 at 37°C within 1 hour.

Quality control

- Long term incubation testing: At the optimal reaction temperature, 10U SspDI was incubated with 1μg pBR322 for 16 hours, and no non-specific degradation of the substrate caused by other nuclease contamination or star activity was detected.
- Enzyme digestion ligation re digestion detection: At the optimal reaction temperature, use 10U SspDI to digest the substrate and recover the enzyme digestion product. Using an appropriate amount of T4 DNA Ligase (Fast) at 22°C can reconnect the enzyme cleavage products. After recycling the connecting product again, the same endonuclease can be used to cleave the connecting product again.

Icon annotation





Homolytic enzyme: KasI, DinI, EgeI, EheI, Mly113I, NarI, PluTI, SfoI

Note: Homolytic enzymes may have different sensitivities to different methylation modifications.

- The optimal reaction temperature is 37°C.
- For DNA methylated by CpG, splicing may be hindered.
- Inactivation condition: Incubate at 80°C 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 ₂ O	up to 15μL
10×FlashCut™ Buffer or 10×FlashCut™ Color Buffer	5μL
Substrate DNA	1μg
SspDI(10U/μL)	1μL
Total	50μL

The DNA substrate should not contain phenol, chloroform, ethanol, EDTA, detergents or high concentration salts, otherwise it will affect the activity of SspDI enzyme.

- Gently suck or tap the tube wall to mix well (without vortexing), then centrifuge instantly to collect hanging wall droplets.
- Incubate at 37°C for 1-16 hours.
- Incubate at 80°C for 20 minutes to inactivate the enzyme and stop the reaction (optional).

2. Note

- 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;
- The additives (such as glycerol, salt) in the storage buffer of 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 of enzyme cleavage reaction;

The number of enzyme cleavage sites in different DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
1	2	3	1	1	0	1	20

The influence of methylation modification

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

Activity in different reaction buffers

	Cut One™ Buffer	Thermo Scientific Fast Digest Buffer	NEB Cut Smart® Buffer	Takara Quick Cut™ Buffer
Reactivity	100%	<12.5%	100%	<25%

Note: The activity data comes from the detection under the restriction enzyme standard reaction system.