SgeI

Cat #: C-BSM1501

Size: 250 U

Storage: -20°C

5'...m^{5}C N N G(N)$_9$▲...3'
3'... G N N C(N)$_{13}$...5'*

*SgeI cleaves DNA targets containing 5-methylcytosine on one or both DNA strands.

Components

Components	Amount
SgeI (5U/μl)	50 μl
10× SgeI Buffer	1 ml

Description

SgeI can cleave DNA targets containing 5-methylcytosine on one or both DNA strands. SgeI restriction endonuclease recognizes the m5CNNG (9/13)^ site and exhibits optimal cleavage efficiency at 37°C in its specific buffer. To ensure consistent performance, its storage buffer contains BSA, which can enhance enzyme stability and bind to potential contaminants in DNA.

Recommended Reaction Conditions

1× SgeI Buffer;

Incubate at 37°C;

Refer to "Protocol for DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80°C for 20 minutes.

Unit Definition

One unit is defined as the amount of SgeI at which no change in the fragmentation pattern is observed with further increase of enzyme. For unit definition 1 µg of pUC19-SgeI DNA (Dcm+) was incubated with the enzyme for 1 hour at 37°C in 50 µL of 1× SgeI Buffer.

Quality Control

Endonuclease residues

Incubate 3U of SgeI with supercoiled plasmid DNA at 37°C for 4 hours, no changes for the plasmid were detected in DNA electrophoresis.

Exonuclease residues

Incubate 5U of SgeI with double-stranded DNA substrate at 37°C for 1 hours, no changes for the substrate were detected in DNA electrophoresis.

Notes

1. The substrate requires at least 2 recognition sequences for SgeI to achieve efficient cleavage.

2. Complete digestion of methylated DNA depends on the number of recognition sites for SgeI. Additionally, the DNA generated by the cleavage of recognition sites can promote non-specific cleavage by SgeI. Therefore, it is recommended to optimize the amount of SgeI used in the digestion reaction.

Icon Descriptions

37 The enzyme's optimum reaction temperature is 37°C.

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

3 hours incubation do not show star activity, but longer incubation may result in star activity.

Method of application

Protocol for Fast DNA Digestion

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

	DNA
ddH_2O	To 20 µl
10× SgeI Buffer	2 µl
DNA	2 µl (0.5-2 µg)
SgeI	0.2-1 µl
Total	20 µl

Note: The total reaction volume can be scaled up or down. The reaction time is not recommended to exceed 1 h.

② Mix gently(Do not vortex) and spin down;

③ Incubate at 37°C for 1 h;

④ Optional: Inactivate the enzyme by heating for 20 minutes at 80°C;

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	Cleave DNA that is methylated by Dcm methyltransferase	Cleave targets that overlap with CpG methylation sequences	No effect	No effect