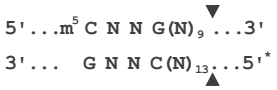
SgeI

REF: EG21501S



*SgeI recognizes and cleaves DNA target sequences containing 5-mC sites, and can be recognized by either one-strand or double-strand methylation.

37 ★

Storage Condition

-20℃

Components

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

Description

SgeI is a restriction endonuclease that cleaves DNA targets containing 5-methylcytosine on either single-stranded or double-stranded DNA. SgeI recognizes the m5CNNG(9/13)^ site and exhibits optimal cleavage in its unique buffer at 37℃ . To ensure consistent performance, the enzyme's storage buffer contains pre-mixed HSA, which enhances enzyme stability and binds to potential contaminants that may be present in DNA.

Recommended Reaction Conditions

1× SgeI Buffer;
Incubate at 37℃ ;
Refer to "Protocol for DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80℃ for 20 minutes.

Definition of Activity Unit

Under 1× SgeI Buffer conditions, 1 μg of pUC19-SgeI DNA (Dcm+) was incubated at 37 ℃ for 1 h in a 50 μl reaction system, increasing the enzyme amount until the digestion product DNA band type does not change with increasing enzyme amount, at which point the enzyme amount is defined as 1 U.

Quality Control Assays

Non-specific Endonuclease Activity

A 20 μl reaction containing 1 μg of supercoiled plasmid and 3 U of SgeI incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 5 U of SgeI incubated for 1 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Notes

1. At least two SgeI recognition sequences are required on the substrate for effective enzyme cleavage.

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

Icon Descriptions

37 The enzyme's optimum reaction temperature is 37℃ .

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

★ 3 hours incubation did not show star activity, but delayed enzyme digestion might show star activity.

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Protocol

1. Protocol for DNA Digestion

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

Reagents	Volume
ddH_2O	to 20 µl
10× Sgel Buffer	2 µl
DNA	2 µl (0.5~2 µg)
SgeI	0.2~1 µl
Total	20 µl

Note: The reaction system can be scaled up or down proportionally. It is not recommended to exceed a reaction time of 1 hour.

② Mix gently and spin down;
③ Incubate at 37℃ for 1 hour;
④ Optional: Inactivate the enzyme by heating for 20 minutes at 80℃ ;

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	Always cleave DNA methylated by Dcm methyltransferase	Cutting target sites that overlap with CpG methylation sequences	No effect	No effect

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