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SgeI

Product Number: RE0579

Shipping and Storage

-20℃.

Components

Component	Specifications
SgeI (5U/μL)	50μL
10×SgeI Buffer	1mL

Description

SgeI can cleave DNA targets containing 5-methylcytosine on single or double stranded DNA. SgeI restriction endonuclease can recognize the m5CNNG (9/13) ^ site and has the best cleavage effect at 37°C in its unique buffer. To ensure consistent performance, the enzyme storage buffer contains pre mixed BSA, which enhances enzyme stability and binds to potential contaminants in DNA preparations.

Suggested reaction conditions

1. 1×SgeI buffer solution;
2. Incubate at 37℃;
3. Prepare the reaction system according to the "DNA enzyme digestion process".

Inactivation conditions

Incubate at 80℃ for 20 minutes.

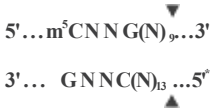
Definition of enzyme activity

Under the condition of 1 × SgeI Buffer, Incubate 1μg pUC19 SgeI DNA (Dcm+) in a 50μL reaction system at 37℃ for 1 hour, continuously increasing the enzyme amount until the DNA band pattern of the enzyme cleavage product does not change with the increase of enzyme amount. At this point, the enzyme amount is defined as 1 U.

Quality control

1. Detection of residual endonuclease: Incubate 3U SgeI with supercoiled plasmid DNA at 37℃ for 4 hours, and detect no changes in the plasmid by DNA electrophoresis.
2. Detection of residual nucleases: Incubate 5U SgeI with double stranded DNA substrate at 37℃ for 1 hour, and detect no change in the double stranded DNA substrate by DNA electrophoresis.

Icon annotation



***SgeI can recognize and cleave DNA target sequences containing 5-mC sites, and can recognize single strand or double strand methylation.**

1. 37 The optimal reaction temperature is 37℃.

2. Inactivation condition: Incubate at 80℃ for 20 minutes.
3. 3 hours of incubation did not show star activity, and longer enzyme digestion may result in star activity.

Protocol

1. DNA enzyme digestion process

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

	DNA
ddH_2O	to 20μL
10× Sgel Buffer	2μL
Substrate DNA	2μL(0.5- 2μg)
Sgel	0.2-1μL
Total	20μL

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

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 37℃ for 1 hour;

1.4. Incubate at 80℃ for 20 minutes to inactivate the enzyme and stop the reaction (optional).

The influence of methylation modification

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	Always cutting DNA methylated by Dcm methyltransferase	Cut targets that overlap with CpG methylation sequences	no effect	no effect