Thermo SCIENTIFIC

PRODUCT INFORMATION

SgeI

#ER2211 250 U

Lot: ___ **Expiry Date: _**

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

Concentration: 3 U/µL
Supplied with: 1 mL of 10X Buffer SgeI

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer SgeI

10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 100 mM KCl, 0.02% Triton X-100, 0.1 mg/mL BSA.

Incubation temperature

37°C.

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 pBR322 DNA isolated from *E.coli dcm*$^+$ strain was incubated with the enzyme for 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double digests

SgeI is not recommended for double digestions.

Storage Buffer

SgeI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM NaCl, 1 mM DTT, 1 mM EDTA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer SgeI	2 µL
DNA (0.5-2 µg/µL)	1 µL
SgeI	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1 hour.

The digestion reaction may be scaled either up or down. Digestion for more than 1 hour is not recommended.

Thermal Inactivation

SgeI is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

SgeI	B	G	O	R	Tango	2X Tango
100	0-20	0-20	0-20	NR*	NR*	NR*

* nonspecific cleavage appears prior to complete digestion of DNA with SgeI.

Star Activity

Greater than 3-fold overdigestion with SgeI may result in nonspecific cleavage.

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: always cleaves DNA methylated by Dcm methyltransferase.
CpG: cleaves targets overlapping with CpG methylated sequences.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

Digestion of 1 µg of DNA for 16 hours at 37°C with 0.1 unit of SgeI results in star activity of the enzyme. Prolonged incubation is not recommended.

Digestion of Agarose-embedded DNA

A minimum of 3 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded pBR322 DNA isolated from *E.coli dcm*$^+$ strain DNA in 16 hours at 37°C.

For **CERTIFICATE OF ANALYSIS** *see* back page

Notes

- At least two copies of SgeI recognition site are required for an efficient cleavage.
- Amount of the enzyme required for complete digestion of methylated DNA depends on the number of SgeI recognition sites. DNA cleavage products generated by target site cleavage facilitate the nonspecific cleavage by SgeI. Therefore, optimization of enzyme amount is recommended for DNA cleavage.
- pBR322 DNA isolated from *E.coli dcm*$^+$ strain (#SD0041) can be used as a DNA cleavage efficiency control. SgeI cleaves all six dcm methylated targets on pBR322 DNA.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern of pBR322 DNA isolated from *E.coli dcm*$^+$ strain is observed after 3-fold overdigestion (3 U/µg pBR322 DNA x 1 hour) with SgeI (*see* Star Activity).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of SgeI for 4 hours.

Quality authorized by: Jurgita Zilinskiene

Note:
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PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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