

## PRODUCT INFORMATION

# SgeI

**#ER2211** 250 u

**Lot:**                      **Expiry Date:**



\* 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/fermentas](http://www.thermoscientific.com/fermentas)

## RECOMMENDATIONS

### 1X Buffer SgeI

10 mM Tris-HCl (pH 8.0), 5 mM MgCl<sub>2</sub>, 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*<sup>+</sup> 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 $\mu$ l
10X Buffer Sgel	2 $\mu$ l
DNA (0.5-2 $\mu$ g/ $\mu$ l)	1 $\mu$ l
Sgel	0.5-2 $\mu$ l
- 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

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

## ENZYME PROPERTIES

### Enzyme Activity in Thermo Scientific REase Buffers, %

Sgel	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 Sgel.

## Star Activity

Greater than 3-fold overdigestion with Sgel 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  $\mu$ g of DNA for 16 hours at 37°C with 0.1 unit of Sgel 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  $\mu$ g of agarose-embedded pBR322 DNA isolated from *E.coli dcm*<sup>+</sup> strain DNA in 16 hours at 37°C.

## Notes

- At least two copies of Sgel recognition site are required for an efficient cleavage.
- Amount of the enzyme required for complete digestion of methylated DNA depends on the number of Sgel recognition sites. DNA cleavage products generated by target site cleavage facilitate the nonspecific cleavage by Sgel. Therefore, optimization of enzyme amount is recommended for DNA cleavage.
- pBR322 DNA isolated from *E.coli dcm*<sup>+</sup> strain (#SD0041) can be used as a DNA cleavage efficiency control. Sgel 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*<sup>+</sup> strain is observed after 3-fold overdigestion (3 u/μg pBR322 DNA x 1 hour) with Sgel (*see* Star Activity).

### Ligation/Recutting Assay

After a 2-fold overdigestion (2 u/μg pBR322 DNA isolated from *E.coli dcm*<sup>+</sup> strain x 1 hour) with Sgel, more than 80% of the digested pBR322 DNA fragments can be ligated at a 5'-termini concentration of 0.6 μM. More than 80% of these sites can be recut.

### Labeled Oligonucleotide (LO) Assay

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

Quality authorized by:

 Jurgita Zilinskiene

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