Thermo SCIENTIFIC

PRODUCT INFORMATION

SfiI

#ER1821 1000 U

Lot: ___ **Expiry Date: _**

5'...**G G C C N N N N↓N G G C C**...3'
3'...**C C G G N↑N N N N C C G G**...5'

Concentration: 10 U/µL
Supplied with: 1 mL of 10X Buffer G
1 mL of 10X Buffer Tango

Store at -20°C

BSA included
www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer G (for 100% SfiI digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
50°C*****.

Unit Definition
One unit is defined as the amount of SfiI required to digest 1 µg of control DNA in 1 hour at 50°C in 50 µL of recommended reaction buffer. The control DNA is pUC19 DNA with inserted SfiI recognition sites-SspI fragments.

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
Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.
1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

***** Incubation at 37°C results in 10% activity.

Storage Buffer

SfiI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 300 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, 0.15% Triton X-100, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

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

- Mix gently and spin down for a few seconds.
- Incubate at 50°C for 1-16 hours.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µL (about 1 µg of DNA)
Water, nuclease-free	16 µL
10X Buffer G	2 µL
SfiI	1-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 50°C for 1-16 hours.

Thermal Inactivation

SfiI is not inactivated by incubation at 80°C for 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
  – stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
  – extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
  – dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
  – check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **CERTIFICATE OF ANALYSIS** *see* back page

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
50-100	**100**	20-50	0-20	100	0-20

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: may overlap – cleavage impaired.
CpG: may overlap – cleavage impaired.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1 µg of Ad2 DNA in 16 hours at 50°C.

Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded Ad2 DNA in 16 hours.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19	Ad2
0	0	0	0	0	0	0	3

Note

- SfiI cleavage is impaired by overlapping *dcm* methylation. To avoid *dcm* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).
- For cleavage with SfiI at least two copies of its recognition sequence are required. The two sites can be on either the same or different DNA molecules (Wertzell, L.M. et al., J. Mol. Biol., 248, 581-595, 1995). Therefore also an oligonucleotide harboring a SfiI recognition site can be supplemented.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with SfiI (10 U/µg pUC19-SfiI DNA x 16 hours).

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 SfiI for 4 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test

Quality authorized by:  Jurgita Zilinskiene

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.