Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

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 G G C C**...5'

Concentration: 10 u/µl
Source: *Streptomyces fimbriatus*
Supplied with: 1 ml of 10X Buffer G
1 ml of 10X Buffer Tango™

Store at -20°C

In total 3 vials. BSA included: Lot# BSA62-313P

ISO 9001 ISO 14001
www.fermentas.com

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 Ad2 DNA in 1 hour at 50°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
Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to www.fermentas.com/doubledigest to choose the best buffer for your experiments.
1X Tango™ Buffer:
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

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* 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 inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Fermentas 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

- Assayed on Adenovirus-2 DNA.
- For cleavage with SfiI at least two copies of its recognition sequence are required.

For **QUALITY CONTROL ASSAY DATA** *see* back page

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with SfiI (10 u/µg Ad2 DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with SfiI, more than 95% of the DNA fragments can be ligated at a 5'-termini concentration of 0.05 µM. More than 95% 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 SfiI for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of SfiI for 16 hours. After religation and transformation, the background level of white colonies was 0.4%.

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.fermentas.com for Material Safety Data Sheet of the product.

(5) Revised 18.09.2006