

PRODUCT INFORMATION

SfaAI (AsiSI)

#ER2091 1000 U

Lot: ____ **Expiry Date:** __

5'...**G C G A T↓C G C**...3'
3'...**C G C↑T A G C G**...5'

Concentration: 10 U/μL
Source: *E.coli* that carries cloned *sfaAIR* gene
 from *Sphingomonas faenia* RFLI
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% SfaAI digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of SfaAI required to digest 1 μg of control DNA in 1 hour at 37°C in 50 μL of recommended reaction buffer. The control DNA is linearized pJET1 DNA with inserted SfaAI recognition site.

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 provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer

SfaAI is supplied in: 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.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 μ L
10X Buffer Tango	2 μ L
DNA (0.5-1 μ g/ μ L)	1 μ L
SfaAI	0.5-2 μ L
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 μ L (~0.1-0.5 μ g of DNA)
nuclease-free water	18 μ L
10X Buffer Tango	2 μ L
SfaAI	1-2 μ L
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

SfaAI is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

Dam: completely overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: completely overlaps – blocked.

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 μ g of pJET1 DNA with inserted SfaAI recognition sequence in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 μ g of agarose-embedded pJET1 DNA with inserted SfaAI recognition sequence in 16 hours.

Compatible Ends

Bsh1285I, PaeI, PvuI.

Number of Recognition Sites in DNA

Ad2	λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
1	0	0	0	0	0	0	0

For **CERTIFICATE OF ANALYSIS** see back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with SfaAI (10 U/μg 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 SfaAI 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.

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