Thermo SCIENTIFIC

PRODUCT INFORMATION

SspI

#____ ____

Lot: ___ Expiry Date: _

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

Concentration:	10 u/µl
Source:	*Sphaerotilus species*
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% SspI digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/ml BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of SspI required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µl of recommended reaction buffer.

Dilution
Dilute with the 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 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.

Storage Buffer

SspI 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 µl
10X Buffer G	2 µl
DNA (0.5-1 µg/µl)	1 µl
SspI	0.5-1 µl*

- 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 µl (~0.1-0.5 µg of DNA)
nuclease-free water	18 µl
10X Buffer G	2 µl
SspI	1-2 µl*

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

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* *See* Star Activity.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Star Activity

An excess of SspI (20 u/µg DNA x 1 hour) may result in star activity.

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of lambda DNA 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 µg of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
20	1	1	1	1	2	6

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

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 15-fold overdigestion with SspI (15 u/µg lambda DNA x 1 hour) (*see* Star Activity).

Ligation/Recutting Assay

After a 10-fold overdigestion (2 u/µg DNA x 5 hours) with SspI, more than 90% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.3 µM. More than 90% 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 SspI for 4 hours.

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.