Thermo SCIENTIFIC

PRODUCT INFORMATION

SmaI

#ER0665 2000 U

Lot: ___ **Expiry Date: _**

5'…**C C C↓G G G**…3'
3'…**G G G↑C C C**…5'

Concentration: 10 U/µL
Source: *Serratia marcescens*
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C

In total 2 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

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

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

Incubation temperature

30°C*****.

Unit Definition

One unit is defined as the amount of SmaI required to digest 1 µg of lambda DNA-Eco81I fragments in 1 hour at 30°C in 50µL 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 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

SmaI 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.

***** Incubation at 37°C results in 50% activity.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
SmaI	0.5-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 30°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 Tango	2 µL
SmaI	1-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 30°C for 1-16 hours.

** This volume of the enzyme is recommended for preparations of standard concentrations (10 U/µL), whereas HC enzymes (50 U/µL) should be diluted with Dilution Buffer to obtain 10 U/µL concentration.

Thermal Inactivation

SmaI 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
50-100	0-20	0-20	0-20	**100**	0-20

Methylation Effects on Digestion

Dam: never 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 µg of lambda DNA in 16 hours at 30°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
3	0	0	1	1	1	1

Note

- Incubation at 37°C results in 50% activity. SmaI has a half-life of 15 min at 37°C.
- Incubation at 25°C results in 100% activity.
- SmaI needs K^+ to work for activity.

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 SmaI (10 U/µg lambda DNA × 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 SmaI 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.