Thermo s c i e n t i f i c

PRODUCT INFORMATION

Smal

#ER0662 5x1200 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:	2x1 mL of 10X Buffer Tango

Store at -20°C



In total 7 vials.

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% Smal 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 Smal required to digest 1 μ g of lambda DNA-Eco81I fragments in 1 hour at 30°C in 50 μ L 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

of Tango Buffer. Please go to

<u>www.thermoscientific.com/doubledigest</u> to choose the best buffer for your experiments.

Storage Buffer

Smal 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:

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nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 μg/μL)	1 µL
Smal	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
Smal	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/ μ L), whereas HC enzymes (50 U/ μ L) should be diluted with Dilution Buffer to obtain 10 U/ μ L concentration.

Thermal Inactivation

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

Rev.9

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

В	G	0	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 μ g 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 μ g of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
3	0	0	1	1	1	1
Note						
 Incubation at 37°C results in 50% activity. Small has 						

- Incubation at 37°C results in 50% activity. Smal has a half-life of 15 min at 37°C.
- Incubation at 25°C results in 100% activity.
- Smal needs $\mathsf{K}^{\scriptscriptstyle +}$ to work for activity.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Smal (10 U/ μ g lambda DNA \times 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of Smal 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 <u>www.thermoscientific.com/onebio</u> for Material Safety Data Sheet of the product.

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