

#### PRODUCT INFORMATION

## SmiI (SwaI)

**#ER1241** 1000 U

Lot: \_\_\_ Expiry Date: \_

5'...**A T T T<sup>↓</sup>A A A T**...3'

3'...**T A A A**↑**T T T A**...5'

Concentration: 10 U/µL

Source: Streptococcus milleri S Supplied with: 1 mL of 10X Buffer 0

1 mL of 10X Buffer Tango

#### Store at -20°C









In total 3 vials.

BSA included

#### www.thermoscientific.com/onebio

## **RECOMMENDATIONS**

**1X Buffer 0** (for 100% Smil digestion)
50 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 100 mM NaCl, 0.1 mg/mL BSA.

## **Incubation temperature**

30°C\*.

#### **Unit Definition**

One unit is defined as the amount of Smil required to digest 1  $\mu$ g of Ad2 DNA-Sspl fragments in 1 hour at 30°C in 50  $\mu$ 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**

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 <a href="https://www.thermoscientific.com/doubledigest">www.thermoscientific.com/doubledigest</a> 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.

<sup>\*</sup> Incubation at 37°C results in 70% activity.



## **Storage Buffer**

Smil is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

## **Recommended Protocol for Digestion**

Add:

- 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  $\mu$ L (~0.1-0.5  $\mu$ g of DNA) nuclease-free water 18  $\mu$ L 10X Buffer 0 2  $\mu$ L Smil 1-2  $\mu$ L

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

#### **Thermal Inactivation**

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

#### **ENZYME PROPERTIES**

## **Enzyme Activity in Thermo Scientific REase Buffers, %**

В	G	0	R	Tango	2X Tango
0-20	0-20	100	20-50	0-20	20-50

## **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 – effect not determined.

## **Stability during Prolonged Incubation**

A minimum of 0.1 units of the enzyme is required for complete digestion of 1  $\mu$ g of Ad2 DNA in 16 hours at 30°C.

## **Digestion of Agarose-embedded DNA**

A minimum of 10 units of the enzyme is required for complete digestion of 1  $\mu$ g of agarose-embedded Adenovirus-2 DNA in 16 hours.

## **Number of Recognition Sites in DNA**

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19	Ad2
0	0	0	0	0	0	1	1

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 SmiI (10 U/ $\mu$ g Ad2 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of Smil 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 <a href="https://www.thermoscientific.com/onebio">www.thermoscientific.com/onebio</a> for Material Safety Data Sheet of the product.

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