



## CERTIFICATE OF ANALYSIS

# SacI

#ER1133 HC, 6000 u

Lot: Expiry Date:

5'...G A G C T↓C...3'

3'...C↑T C G A G...5'

Concentration: 50 u/μl  
Source: *Streptomyces achromogenes*  
Supplied with: 2x1 ml of 10X Buffer SacI  
1 ml of 10X Buffer Tango™

Store at -20°C



In total 4 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer SacI** (for 100% SacI digestion)

10 mM Bis-Tris Propane-HCl (pH 6.5), 10 mM MgCl<sub>2</sub>,  
0.1 mg/ml BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of SacI required to digest 1 μg of lambda DNA-HindIII fragment in 1 hour at 37°C in 50 μl 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 is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to [www.fermentas.com/doubledigest](http://www.fermentas.com/doubledigest) to choose the best buffer for your experiments.

1X Tango™ Buffer:

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

## Storage Buffer

SacI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM DTT, 1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer SacI	2 µl
DNA (0.5-1 µg/µl)	1 µl
SacI	0.5-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

## Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µl (about 1 µg of DNA)
nuclease-free water	16 µl
10X Buffer SacI	2 µl
SacI	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°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

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

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

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

### Methylation Effects on Digestion

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

### Compatible Ends

Alw21I, Eco24I, SduI

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
2	0	0	1	1	1	1

For **QUALITY CONTROL ASSAY DATA** see back page

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion (10 u/μg lambda DNA x 16 hours) with SacI.

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with SacI, more than 95% of the digested M13mp18 DNA fragments can be ligated at a 5'-termini concentration of 0.04 μM. More than 95% 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 SacI for 4 hours.

## Blue/White Cloning Assay

pUC57 was incubated with 10 units of SacI for 16 hours. After religation and transformation the background level of white colonies was 0.3%.

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.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.