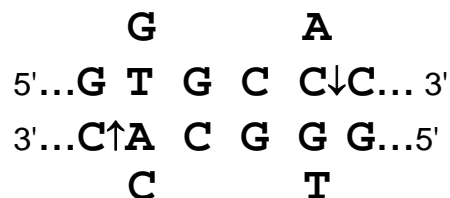


CERTIFICATE OF ANALYSIS

BseSI (Bme1580I)

#ER1442 2500 u

Lot: Expiry Date:



Concentration: 10 units/μl
 Source: *Bacillus stearothermophilus* Jo 10-553
 Supplied with: 1 ml of 10X Buffer G
 1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer G (for 100% BseSI digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl, 0.1 mg/ml BSA.

Incubation temperature

55°C*.

Unit Definition

One unit is defined as the amount of BseSI required to digest 1 μg of lambda DNA in 1 hour at 55°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 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.

* Incubation at 37°C results in less than 20% activity.

Storage Buffer

BseSI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 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
BseSI	0.5-2 µl
- Mix gently and spin down for a few seconds.
- Incubate at 55°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
BseSI	1-2 µl
- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours.

Thermal Inactivation

Only small amounts of BseSI (up to 10 units) can be inactivated at 80°C in 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
 - stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
 - extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
 - dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
 - check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **QUALITY CONTROL ASSAY DATA**
see back page

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

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

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: may overlap – no effect.

CpG: may overlap – no effect.

EcoKI: may overlap – cleavage impaired.

EcoBI: never overlaps – 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 55°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

GGGCC↓C – ApaI, Eco24I, SduI

GTGCA↓C – Alw21I, Mph1103I, PstI, SdaI, SduI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
10	1	3	4	3	2	2*

** According to our experimental data, BseSI has only one recognition site of a position 2088.

QUALITY CONTROL ASSAY DATA

Overdigestion Assay


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

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with BseSI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.1 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 BseSI 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.fermentas.com for Material Safety Data Sheet of the product.