

CERTIFICATE OF ANALYSIS

BshTI (AgeI)

#ER1461 200u

Lot: Expiry Date:

5'...**A**↓**C C G G T**...3'
3'...**T G G C C**↑**A**...5'

Concentration: 10 units/μl
Source: *Bacillus sphaericus* Jo 22-024
Supplied with: 1ml of 10X Buffer O
 1ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer O (for 100% BshTI digestion)

50mM Tris-HCl (pH 7.5), 10mM MgCl₂, 100mM NaCl,
0.1mg/ml BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of BshTI required to digest 1μg of lambda DNA in 1 hour at 37°C in 50μl of recommended reaction buffer.

Dilution

Dilute with Dilution Buffer (#B19): 10mM Tris-HCl (pH 7.4 at 25°C) 100mM KCl, 1mM EDTA, 1mM DTT, 0.2mg/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:

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

Storage Buffer

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

Recommended Protocol for Digestion

- Add:

nuclease-free water	16µl
10X Buffer O	2µl
DNA (0.5-1µg/µl)	1µl
BshTI	0.5-2µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°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	10µl (~0.1-0.5µg of DNA)
nuclease-free water	18µl
10X Buffer O	2µl
BshTI	1-2µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

* See Note.

Thermal Inactivation

BshTI is inactivated by incubation at 80°C for 20min.

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

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

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked.
EcoKI: may overlap – effect not determined.
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 1µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 20 units of the enzyme is required for complete digestion of 1µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

Cfr10I, Kpn2I, Cfr9I, Eco88I (C↓CCGGG), BsaWI, SgrAI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
13	0	0	0	0	0	0

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 80-fold overdigestion with BshTI (5u/ μ g lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3u/ μ g DNA x 17 hours) with BshTI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.13 μ M. More than 95% of these 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 BshTI for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of BshTI for 1 hour. After religation and transformation, the background level of white colonies was 0.2%.

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.