

CERTIFICATE OF ANALYSIS

BstXI

#ER1021 500 u

Lot: **Expiry Date:**

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

Concentration: 10 u/μl
Source: *Bacillus stearothermophilus* X
Supplied with: 1 ml of 10X Buffer O
 1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer O (for 100% BstXI digestion)

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

Incubation temperature

55°C*.

Unit Definition

One unit is defined as the amount of BstXI 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 50% activity.

Storage Buffer

BstXI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 μ l
10X Buffer O	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
BstXI	0.5-2 μ l*
- 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 μ l (~0.1-0.5 μ g of DNA)
nuclease-free water	18 μ l
10X Buffer O	2 μ l
BstXI	1-2 μ l*
- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours*.

* See Star Activity.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

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

Star Activity

An excess of BstXI (20 u/ μ g DNA x 1 hour) may result in star activity.

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.1 unit of the enzyme is required for complete digestion of 1 μ g 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 μ g of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
13	3	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 15-fold overdigestion with BstXI (15 u/μg lambda DNA x 1 hour) (see Star Activity).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with BstXI, approximately 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.13 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 BstXI for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of BstXI for 16 hours. After religation and transformation, the background level of white colonies was 0.4%.

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.