

PRODUCT INFORMATION

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



BSA included

www.thermoscientific.com/onebio

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

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 www.thermoscientific.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 µL
10X Buffer O	2 µL
DNA (0.5-1 µg/µL)	1 µL
BstXI	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 O	2 µL
BstXI	1-2 µL**
- 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 Thermo Scientific 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/µg 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 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.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
13	3	0	0	0	0	0

Note

BstXI cleavage is impaired by overlapping *dcm* methylation. To avoid *dcm* methylation, use a *dam*⁻, *dcm*⁻ strain such as GM2163 (#M0099).

For **CERTIFICATE OF ANALYSIS** see back page

CERTIFICATE OF ANALYSIS

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 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 double-stranded labeled oligonucleotides occurred during incubation with 10 units of BstXI 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 www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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