

CERTIFICATE OF ANALYSIS

# BseJI (BsaBI)

#ER1711      2000 u

Lot:                      Expiry Date:

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

Concentration:      10 u/μl  
Source:                *Bacillus stearothermophilus* Tsp5  
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% BseJI digestion)

50 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 100 mM NaCl,  
0.1 mg/ml BSA.

**Incubation temperature**

65°C\*.

**Unit Definition**

One unit is defined as the amount of BseJI required to digest 1 μg of lambda DNA in 1 hour at 65°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 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

\* Incubation at 37°C results in less than 10% activity.

## Storage Buffer

BseJI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM NaCl, 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 O	2 µl
DNA (0.5-1 µg/µl)	1 µl
BseJI	0.5-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 65°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
BseJI	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 65°C for 1-16 hours\*.

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\* See Star Activity on back page.

## Thermal Inactivation

BseJI is not inactivated by incubation at 80°C for 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™
NR	100**	100	NR	NR	100**

\*\*Star activity appears at a greater than 5-fold overdigestion (5 u x 1h).

NR: buffer is not recommended, because of high star activity.

### Star Activity

An excess of BseJI (20 u/μg DNA x 1 hour) may result in star activity.

### Methylation Effects on Digestion

Dam: may overlap – blocked.

Dcm: never overlaps – no effect.

CpG: may overlap – no effect.

EcoKI: never overlaps – no effect.

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 1 μg of DNA in 16 hours at 65°C.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
21	2	1	0	0	0	2

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 15-fold overdigestion with BseJI (15 u/μg lambda DNA x 1 hour) (see Star Activity).

## Ligation/Recutting Assay

After a 10-fold overdigestion (5 u/μg DNA x 2 hours) with BseJI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.21 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 BseJI for 4 hours.

## Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of BseJI 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.