

CERTIFICATE OF ANALYSIS

# BspPI (AlwI)

#ER1322      500 u

Lot:                      Expiry Date:

5'...G G A T C (N)<sub>4</sub>↓...3'  
3'...C C T A G (N)<sub>5</sub>↑...5'

Concentration:      2 u/μl  
Source:                *Bacillus species* d1-34  
Supplied with:      1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer Tango™** (for 100% BspPI digestion)  
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

### Incubation temperature

55°C\*.

### Unit Definition

One unit is defined as the amount of BspPI required to digest 1 μg of lambda DNA *dam*<sup>-</sup> 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](http://www.fermentas.com/doubledigest) to choose the best buffer for your experiments.

### Storage Buffer

BspPI is supplied in: 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.

\* Incubation at 37°C results in 30% activity.

## Recommended Protocol for Digestion

- Add:

|                              |               |
|------------------------------|---------------|
| nuclease-free water          | 16 $\mu$ l    |
| 10X Buffer Tango™            | 2 $\mu$ l     |
| DNA (0.5-1 $\mu$ g/ $\mu$ l) | 1 $\mu$ l     |
| BspPI                        | 0.5-2 $\mu$ 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 $\mu$ l (~0.1-0.5 $\mu$ g of DNA) |
| nuclease-free water  | 18 $\mu$ l                           |
| 10X Buffer Tango™    | 2 $\mu$ l                            |
| BspPI                | 1-2 $\mu$ l                          |
- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours.

## Thermal Inactivation

BspPI 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 | 20-50 | 0-20 | 0-20 | 100    | 0-20      |

### Methylation Effects on Digestion

Dam: completely overlaps – blocked.

Dcm: may overlap – 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 0.5 units of enzyme is required for complete digestion of 1  $\mu$ g of lambda DNA in 16 hours at 55°C.

### Number of Recognition Sites in DNA

| $\lambda$ | $\Phi$ X174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|-----------|-------------|--------|-------|----------|----------|------------|
| 58        | 0           | 12     | 10    | 10       | 10       | 4          |

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after an 80-fold overdigestion with BspPI (5 u/ $\mu$ g lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 10-fold overdigestion (2 u/ $\mu$ g DNA x 5 hours) with BspPI, approximately 70% of the digested DNA fragments in a reaction mixture containing 20-40 u of T4 DNA ligase/1  $\mu$ g of fragments can be ligated at a 5'-termini concentration of 0.2  $\mu$ M. More than 50% 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 BspPI 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](http://www.fermentas.com) for Material Safety Data Sheet of the product.