

CERTIFICATE OF ANALYSIS

BspPI (AlwI)

#ER1321 100 u

Lot: Expiry Date:

5'...G G A T C (N)₄↓...3'
3'...C C T A G (N)₅↑...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*⁻ 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.

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 μ l
10X Buffer Tango™	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
BspPI	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 Tango™	2 μ l
BspPI	1-2 μ 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 μ g of lambda DNA in 16 hours at 55°C.

Number of Recognition Sites in DNA

λ	Φ 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/μg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 10-fold overdigestion (2 u/μg 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 μg of fragments can be ligated at a 5'-termini concentration of 0.2 μM. 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 for Material Safety Data Sheet of the product.