Thermo SCIENTIFIC

PRODUCT INFORMATION

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
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (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 provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.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 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
BspPI	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 Tango	2 µL	
BspPI	1-2 µL	

- 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 Thermo Scientific 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 – no effect.

Stability during Prolonged Incubation

A minimum of 0.5 units of enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 55°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
58	0	12	10	10	10	4

Note

BspPI is blocked by overlapping *dam* methylation. To avoid *dam* 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 80-fold overdigestion with BspPI (5 U/µg lambda DNA *dam*$^{-}$ x 16 hours).

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 5 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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.