Thermo SCIENTIFIC

PRODUCT INFORMATION

BplI

#ER1311 100 U

Lot: ___ **Expiry Date: _**

5'...↓ $_8$(N) G A G (N)$_5$ C T C (N)$_{13}$↓...3'
3'...↑ $_{13}$(N) C T C (N)$_5$ G A G (N)$_8$ ↑...5'

Concentration: 5 U/µL
Source: *Bacillus pumilus*
Supplied with: 1 mL of 10X Buffer Tango
0.1 mL of 50X SAM (2.5 mM)

Store at -20°C

In total 3 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

[1X Thermo Scientific Tango Buffer] + SAM* (for 100% BplI digestion)

[33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA] + 0.05 mM S-adenosylmethionine (SAM).

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of BplI at which no change in the fragmentation pattern is observed with further increase of enzyme. 1 µg of lambda DNA-XhoI fragments is incubated with BplI for 1 hour at 37°C in 50 µL of recommended reaction buffer. The cleavage of DNA by BplI is never complete.

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.

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* BplI requires only Mg^{2+} for its activity, but is stimulated by S-adenosylmethionine. 0.05 mM S-adenosylmethionine gives more than a 100-fold increase in BplI activity.

Storage Buffer

BplI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 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 Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
50X SAM	0.4 µL
BplI	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°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	
50X SAM	0.6 µL	
BplI	1-2 µL	

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

BplI is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B_{+SAM}	G_{+SAM}	O_{+SAM}	R_{+SAM}	$Tango_{+SAM}$	$2X\ Tango_{+SAM}$
0-20	20-50	0-20	0-20	**100**	20-50

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.3 units of the enzyme is required for digestion of 1 µg of DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum 10 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
1	0	0	0	0	0	0

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with BplI (10 U/µg lambda DNA 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 10 units of BplI 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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