

CERTIFICATE OF ANALYSIS

BpII

#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: Lot# BSA62-313P

RECOMMENDATIONS

[1X Buffer Tango™] + SAM* (for 100% BpII 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 BpII 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 BpII for 1 hour at 37°C in 50 μl of recommended reaction buffer. The cleavage of DNA by BpII 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 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.

* BpII requires only Mg²⁺ for its activity, but is stimulated by S-adenosylmethionine. 0.05 mM S-adenosylmethionine gives more than 100-fold increase in BpII activity.

Storage Buffer

BpII is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 100 mM KCl, 1 mM DTT, 0.1 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
BpII	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
BpII	1-2 µl
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Fermentas 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 **QUALITY CONTROL ASSAY DATA** see back page

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with BpII (10 u/μg lambda DNA x 16 hours).

Ligation/Recutting Assay


After a 10-fold overdigestion (0.6 u/μg DNA x 17 hours) with BpII, more than 70% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.03 μM. None of these can be recut due to the methylation at the recognition sequence by BpII .

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of BpII for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of BpII for 16 hours. After religation and transformation, the background level of white colonies was 0.5%.

Quality authorized by:  Laima Samaliene

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.