

CERTIFICATE OF ANALYSIS

# Bsp120I (ApaI, *see Note*)

#ER0131 1500 u

Lot: Expiry Date:

5'... G↓G G C C C...3'

3'... C C C G↑G...5'

**Note:** Bsp120I - neoschizomer of ApaI, produces DNA fragments that have a 4-base 5'-extension.

Concentration: 10 u/μl  
Source: *Bacillus subtilis* RFL120  
Supplied with: 1 ml of 10X Buffer B  
1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer B** (for 100% Bsp120I digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of Bsp120I required to digest 1 μg of lambda DNA-Cpol fragments in 1 hour at 37°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.

1X Tango™ Buffer:

33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

## Storage Buffer

Bsp120I 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.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer B	2 µl
DNA (0.5-1 µg/µl)	1 µl
Bsp120I	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 B	2 µl
Bsp120I	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

\* This volume of the enzyme is recommended for preparations of standard concentrations (10 u/µl), whereas HC enzymes (50 u/µl) should be diluted with Dilution Buffer to obtain 10 u/µl concentration.

## Thermal Inactivation

Bsp120I 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™
100	20-50	0-20	20-50	50-100	0-20

### Methylation Effects on Digestion

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

### Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

### Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

### Compatible Ends

CfrI, Eco52I, NotI

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
1	0	0	1	0	0	0

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# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 240-fold overdigestion with Bsp120I (15 u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with Bsp120I, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.01 μM. More than 95% of these sites 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 Bsp120I for 4 hours.

## Blue/White Cloning Assay

pUC57 was incubated with 10 units of Bsp120I for 16 hours. After religation and transformation, the background level of white colonies was 0.1%.

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.