

CERTIFICATE OF ANALYSIS

# BcuI (SpeI)

#ER1251      400 u

**Lot:**                      **Expiry Date:**

5'...**A**↓**C T A G T**...3'

3'...**T G A T C**↑**A**...5'

Concentration:      10 u/μl  
Source:                *Bacillus coagulans* VS 29-022  
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% BcuI digestion)

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

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of BcuI required to digest 1 μg of Ad2 DNA 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, 1mM 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.

**Storage Buffer**

BcuI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 300 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 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
DNA (0.5-1 $\mu$ g/ $\mu$ l)	1 $\mu$ l
Bcul	0.5-2 $\mu$ l
- 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 $\mu$ l (~0.1-0.5 $\mu$ g of DNA)
nuclease-free water	18 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
Bcul	1-2 $\mu$ l
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

## Thermal Inactivation

Bcul is not inactivated by incubation at 80°C for 20 min.

## Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
  - stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
  - extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
  - dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
  - check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **QUALITY CONTROL ASSAY DATA**

see back page

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

B	G	O	R	Tango™	2X Tango™
50-100	50-100	0-20	20-50	100	0-20

### Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: never overlaps – no effect.

EcoKI: may overlap – effect not determined.

EcoBI: may overlap – effect not determined.

### Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 µg of Ad2 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 Adenovirus-2 DNA in 16 hours.

### Compatible Ends

XmaI, Eco130I, NheI, XbaI

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19	Ad2
0	0	0	0	0	0	0	3

## QUALITY CONTROL ASSAY DATA

### Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with BclI (10 u/µg Ad2 DNA x 16 hours).

### Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with BclI, more than 80% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.05 µM. More than 90% 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 BclI for 4 hours.

### Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of BclI for 16 hours. After religation and transformation, the background level of white colonies was 0.4%.

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.