

CERTIFICATE OF ANALYSIS

# Bsp68I (NruI)

#ER0112      4000 u

Lot:                      Expiry Date:

5'...**T C G↓C G A**...3'  
3'...**A G C↑G C T**...5'

Concentration:      10 u/μl  
Source:                *Bacillus megaterium* RFL68  
Supplied with:      2 x 1 ml of 10X Buffer O  
                             1 ml of 10X Buffer Tango™

Store at -20°C



In total 4 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer O** (for 100% Bsp68I digestion)

50 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 100 mM NaCl,  
0.1 mg/ml BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of Bsp68I required to digest 1 μg of lambda 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, 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

Bsp68I is supplied: 10 mM Tris-HCl (pH 7.4 at 25°C), 200 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 mg/ml BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer O	2 µl
DNA (0.5-1 µg/µl)	1 µl
Bsp68I	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 O	2 µl
Bsp68I	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Star Activity.

## Thermal Inactivation

Bsp68I is inactivated by incubation at 65°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

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

### Star Activity

An excess of Bsp68I (20 u/µg DNA x 1 hour) may result in star activity.

### Methylation Effects on Digestion

Dam: may overlap – no effect.

Dcm: never overlaps – no effect.

CpG: completely overlaps – blocked.

EcoKI: never overlaps – no effect.

EcoBI: never overlaps – no effect.

### Stability during Prolonged Incubation

A minimum of 0.2 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.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
5	2	1	1	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 15-fold overdigestion with Bsp68I (15 u/μg lambda DNA x 1 hour) (see Star Activity).

## Ligation/Recutting Assay

After a 10-fold overdigestion (0.6 u/μg DNA x 17 hours) with Bsp68I, more than 80% of the digested DNA fragments can be ligated in a reaction mixture containing 20-40 u of T4 DNA ligase/1μg of fragments and 10% PEG at a 5'-termini concentration of 0.06 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 Bsp68I for 4 hours.

## Blue/White Cloning Assay

pUC57 was incubated with 10 units 10 units of Bsp68I for 16 hours. After religation and transformation, the background level of white colonies was 0.14%.

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.