Thermo SCIENTIFIC

PRODUCT INFORMATION

BfmI (SfcI)

#ER1161 200 u

Lot: **Expiry Date:**

5'…**C↓T R Y A G**…3'
3'…**G A Y R T↑C**…5'

Concentration: 10 u/µl
Source: *Bacillus firmus* S8-336
Supplied with: 1 ml of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/fermentas

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% BfmI 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 BfmI 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 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.fermentas.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer
BfmI 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
BfmI	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
BfmI	1-2 µl*

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

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* *See* Overdigestion Assay.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 1.0 unit of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Compatible Ends

C↓TGCAG - Alw44I

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
38	6	4	4	4	6	7

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

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with BfmI (5 u/µg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with BfmI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.5 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 BfmI for 4 hours.

Quality authorized by:  Jurgita Zilinskiene