

PRODUCT INFORMATION

Bpu10I

#ER1181 200 U

Lot: ____ Expiry Date: _

5'...C $C\downarrow T$ N A G C...3' 3'...G G A N $T\uparrow C$ G...5'

Concentration:5 U/µLSource:*E.coli* that carries the cloned *bpu10IR*
gene from *Bacillus pumilus* RFL10Supplied with:1 mL of 10X Buffer Bpu10I
1 mL of 10X Buffer Tango

Store at -20°C



BSA included

Unique

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer Bpu10I (for 100% Bpu10I digestion) 10 mM Bis-Tris Propane-HCI (pH 6.5), 10 mM MgCl₂, 100 mM KCI, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Bpu10I required to digest 1 μ g of lambda DNA in 1 hour at 37°C in 50 μ L 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

Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango[™] Buffer. Please refer to <u>www.thermoscientific.com/doubledigest</u> 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.

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Storage Buffer

Bpu10I is supplied in: 10 mM Tris-HCI (pH 7.5 at 25°C), 200 mM KCI, 1 mM DTT, 0.1mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

• Add:

nuclease-free water	16 µL
10X Buffer Bpu10I	2 µL
DNA (0.5-1 μg/μL)	1 µL
Bpu10I	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:

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

* See Note and Overdigestion Assay.

Thermal Inactivation

Bpu10I is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

Bpu10l	В	G	0	R	Tango	2X Tango
100	0-20	20-50**	50-100**	100**	50-100**	100**

**Star activity appears at a greater than 5-fold overdigestion (5 U x 1h).

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: may overlap – no effect.

Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1 μ g of lambda DNA in 16 hours at 37°C.

Compatible Ends

Bpu1102I, HpyF3I, Eco81I.

Number of Recognition Sites in DNA

λ	Ф Х174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
19	7	1	0	0	0	4

Note

- For cleavage with Bpu10I at least two copies of its recognition sequence are required.
- A large excess of Bpu10I (>4 U/1 µg DNA) may result in incomplete DNA cleavage. Therefore, we recommend increasing the incubation time instead of using an excess of Bpu10I.

For **CERTIFICATE OF ANALYSIS** see back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 48-fold overdigestion with Bpu10I (3 U/ μ g lambda DNA x 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or doublestranded labeled oligonucleotides occurred during incubation with 10 units of Bpu10I for 4 hours.

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 <u>www.thermoscientific.com/onebio</u> for Material Safety Data Sheet of the product.

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