Thermo s c i e n t i f i c

PRODUCT INFORMATION

 BpiI (BbsI)

 #ER1012
 1000 U

 Lot:
 Expiry Date:

 5'...G A A G A C (N) $_2 \downarrow ...3'$

 3'...C T T C T G (N) $_6 \uparrow ...5'$

Concentration:10 U/µLSource:Bacillus pumillus Sw 4-3Supplied with:1 mL of 10X Buffer G1 mL of 10X Buffer Tango

Store at -20°C

37°



BSA included

G

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer G (for 100% Bpil digestion)
10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Bpil 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

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.

Storage Buffer

Bpil 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 G	2 µL
DNA (0.5-1 μg/μL)	1 µL
Bpil	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.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

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 – effect not determined.

Stability during Prolonged Incubation

A minimum of 0.3 units of the enzyme is required for complete digestion of 1 μ g of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

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

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
24	3	3	0	0	0	0

For **CERTIFICATE OF ANALYSIS** see back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Bpil (10 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 Bpil for 4 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

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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