

#### PRODUCT INFORMATION

# **BamHI**

**#ER0051** 4000 U

Lot: \_\_\_ Expiry Date: \_\_\_

5'...**G↓G A T C C**...3'

3'...**C C T A G**↑**G**...5'

Concentration: 10 U/µL

Source: Bacillus amyloliquefaciens H
Supplied with: 2x1 mL of 10X Buffer BamHI

1 mL of 10X Buffer Tango

Store at -20°C











In total 4 vials.

BSA included

www.thermoscientific.com/onebio

#### RECOMMENDATIONS

**1X Buffer BamHI** (for 100% BamHI digestion) 10 mM Tris-HCI (pH 8.0), 5 mM MgCl<sub>2</sub>, 100 mM KCI, 0.02% Triton X-100, 0.1 mg/mL BSA.

# **Incubation temperature**

37°C.

#### **Unit Definition**

One unit is defined as the amount of BamHI required to digest 1  $\mu$ g of lambda DNA-Bsp120I fragments in 1 hour at 37°C in 50  $\mu$ 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 <a href="https://www.thermoscientific.com/doubledigest">www.thermoscientific.com/doubledigest</a> 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.

Rev.13

# **Storage Buffer**

BamHI is supplied in: 10 mM Tris-HCI (pH 7.4 at 25°C), 200 mM NaCI, 1 mM DTT, 0.1 mM EDTA, 0.15% Triton X-100, 0.2 mg/mL BSA and 50% glycerol.

# **Recommended Protocol for Digestion**

• Add:

nuclease-free water 16  $\mu$ L 10X Buffer BamHl 2  $\mu$ L DNA (0.5-1  $\mu$ g/ $\mu$ L) 1  $\mu$ L BamHl 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 2  $\mu$ L BamHI 1-2  $\mu$ L\*,\*\*

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

#### **Thermal Inactivation**

Only small amounts of BamHI (up to 10 units) can be inactivated at 80°C in 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 **CERTIFICATE OF ANALYSIS** see back page

<sup>\*</sup> This volume of the enzyme is recommended for preparations of standard concentrations (10 U/μL), whereas HC enzymes (50 U/μL) should be diluted with Dilution Buffer to obtain 10 U/μL concentration.

<sup>\*\*</sup> See Overdigestion Assay.

#### **ENZYME PROPERTIES**

#### **Enzyme Activity in Thermo Scientific REase Buffers, %**

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

<sup>\*\*</sup>Star activity appears at a greater than 5-fold overdigestion (5 U  $\times$  1h).

# **Methylation Effects on Digestion**

Dam: completely overlaps – no effect.

Dcm: may overlap – no effect. CpG: may overlap – no effect. EcoKI: never overlaps – no effect. EcoBI: never overlaps – no effect.

# **Stability during Prolonged Incubation**

A minimum of 0.5 units of the enzyme is required for complete digestion of 1  $\mu$ g 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  $\mu$ g of agarose-embedded lambda DNA in 16 hours.

# **Compatible Ends**

BcII, BgIII, Bsp143I, MboI, Psul

# **Number of Recognition Sites in DNA**

λ	ФХ174	•			pTZ19R/U	M13mp18/19
5	0	1	1	1	1	1

#### **CERTIFICATE OF ANALYSIS**

# **Overdigestion Assay**

No detectable change in the specific fragmentation pattern is observed after an 80-fold overdigestion with BamHI (5 U/µg 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 BamHI 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:** 



#### **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 <a href="https://www.thermoscientific.com/onebio">www.thermoscientific.com/onebio</a> for Material Safety Data Sheet of the product.

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