Thermo SCIENTIFIC

PRODUCT INFORMATION

BclI

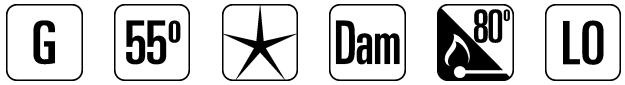
#ER0721 1000 U

Lot: ___ **Expiry Date: _**

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

Concentration:	10 U/µL
Source:	*Bacillus caldolyticus*
Supplied with:	1 mL of 10X Buffer G
	1 mL of 10X Buffer Tango

Store at -20°C



In total 3 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer G (for 100% BclI digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
55°C*****.

Unit Definition
One unit is defined as the amount of BclI required to digest 1 µg of lambda DNA *dam*⁻ in 1 hour at 55°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, 1mM 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 www.thermoscientific.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.

***** Incubation at 37°C results in 50% activity.

Storage Buffer

BclI 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
BclI	0.5-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 55°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 G	2 µL
BclI	1-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours**.

** *See* Star Activity on back page.

Thermal Inactivation

Only small amounts of BclI (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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

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

Star Activity

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.1 units of enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 55°C.

Compatible Ends

BamHI, BglII, Bsp143I, MboI, PsuI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
8	0	0	0	0	0	0

Note

BclI is blocked by overlapping *dam* methylation. To avoid *dam* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 15-fold overdigestion with BclI (15 U/µg lambda DNA *dam*$^-$ x 1 hour) (*see* Star Activity).

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 double-stranded labeled oligonucleotides occurred during incubation with 10 units of BclI 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 www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.