DATA SHEET

BsiS I
(*Hpa* II)

Source: *Bacillus stearothermophilus*

5'…CCGG…3'
3'…GGCC…5'

	Cat.-No.	Size	Conc.
	EN-111S	2,200 units	10 units/µl
	EN-111L	11,000 units	10 units/µl

For *in vitro* use only.
Quality guaranteed for 12 months.
Store at -20°C, avoid frequent thawing and freezing.

Recommended assay

50 µl assay	
5 µl	10x Buffer *Bsi*S I
1-2 µg *or* 10 µl	pure DNA PCR product (~0.1-2 µg DNA)
1-2 units	*Bsi*S I
Fill up to 50 µl	PCR grade water

Use 1 unit/µg DNA, not exceeding 10 % of reaction volume. Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex. High (excess) amounts of enzyme can greatly speed up the reaction. To obtain complete digestion of high molecular weight DNA, (e.g. plant genomic DNA), add excess amounts of enzyme and prolong the incubation time.

Incubate for 5 min. at 55°C.

Stop reaction by alternatively
(a) Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*
(b) Heat Inactivation (No) *or*
(c) Spin Column DNA Purification (e.g.PCR Purification Kit, Cat.-No. PP-201S/L) *or*
(d) Gel Electrophoresis and Single Band Excision (e.g. Agarose Gel Extraction Kit, Cat.-No. PP-202 S/L) *or*
(e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Double Digestion – Buffer Compatibility:

B1 - 25% Relative Activity
B2 - 50 % Relative Activity
B3 - 25 % Relative Activity
B4 - 10-25 % Relative Activity
B5 - 100 % Relative Activity

***BsiS* I**

10 units/µl *Bsi*S I in 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% [v/v] glycerol.

10x Reaction Buffer *BsiS* I

330 mM Tris-acetate (pH 7.9 at 25°C), 100 mM Mg-acetate, 660 mM K-acetate, 5 mM dithiothreitol, 1% Triton X-100 and 1 mg/ml BSA.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

Non-optimal buffer conditions:
To compensate for the lack of enzyme activity, increase the amount of enzyme and / or reaction time accordingly. The following values may serve as orientation:

- → Enzyme amount: Instead of 1 unit of enzyme, use ~4 units in buffers providing 25 % relative activity, ~2 units in 50 %, ~1.5 units in 75 % or ~1 unit in 100 %, respectively.
- → Reaction time: Increase by ~1.3-fold (75 % relative activity), ~2 fold (50 %) or ~4 fold (25 %), respectively.

Reaction Buffer Compatibility:
Both, enzyme and buffers are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

Ligation and recutting:
After 10-fold overdigestion with *Bsi*S I, >95% of the DNA fragments can be ligated and recut with this enzyme.

DNA Methylation:
No Inhibition: dcm, dam, CpG

Unit Definition:
One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA (328 sites) in 1 hour in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Quality Control:
All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/ 5'-phosphatase, as well as nonspecific single- and doublestranded DNase activities.