enzynomics
www.enzynomics.com
info@enzynomics.com

Sfi I

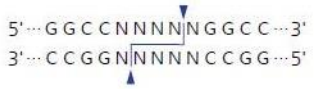
Cat.#	Size	Conc.
R033S	3,500 units	4 units/μl
R033M	7,000 units	4 units/μl
R033L	17,500 units	4 units/μl
R033H	17,500 units	20 units/μl

Expire date:

Store at -20℃

Supplied with: 10X EzBuffer II
10X FastCut Buffer
6X DNA Loading Buffer
Sterile water

Recognition site



For Research Use Only. Not for use in diagnostic procedures.

ISO9001 ISO14001 ISO13485

Source: *Streptomyces fimbriatus*

Reaction conditions
1X EzBuffer II 50℃
1X FastCut Buffer, 50℃

FastCut Buffer
Enzynomics restriction enzyme can cut substrate DNA in 5-15 with FastCut Buffer

1X EzBuffer II
10 mM Tris-HCl (pH 7.9 @ 25℃)
50 mM NaCl
10 mM $MgCl_2$
100 μg/ml BSA

Unit definition
One unit is defined as the amount of enzyme required for complete digestion of 1-μg bacteriophage λ at 50℃ for 1 hr in 50-μl reaction mixtures.

Storage
20 mM Tris-HCl (pH 7.4 @ 25℃), 300 mM NaCl, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 0.15% Triton X-100, 200 μg/ml BSA, 50% glycerol.

Dilution buffer: EzDiluent A
10 mM Tris-HCl (pH 7.4 @ 25℃), 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 μg/ml BSA, 50% glycerol.

Heat Inactivation
No.

Methylation sensitivity
dam methylation: Not sensitive
dcm methylation: Conditionally sensitive
CpG methylation: Conditionally sensitive

Prolonged incubation
A minimum amount of enzyme required to digest 1-μg substrate DNA for 16 hr; 0.25 U.

Relative activity in EzBuffers

EzBuffer I:	25%
EzBuffer II:	100%
EzBuffer III:	25%
EzBuffer IV:	100%
FastCut Buffer:	100%

Note
Cleavage is inhibited by *dcm* methylation and CpG methylation partially overlapping its cleavage site. Better cleavage occurs with long DNA such as chromosomal DNA. It requires two recognition sequences for efficient cleavage by homotetrameric Sfi I. Only 10% activity is obtained at 37℃. It is suitable to generate large DNA fragments from chromosome due to the rare occurrence of the recognition site.

Quality Control Assay

Overdigestion assay : DNA digested for 16 hr in 50-μl reaction with 100 U of enzyme resulted in same the DNA band patterns as those obtained with 1 U of enzyme for 1 hr.

Endonuclease assay: Less than 5% of 1-μg of ΦX174 RFI is converted to RFII when the DNA is incubated with 50 U of enzyme at 37℃ for 4hr in 50-μl reaction.

Ligation and recutting: More than 95% of DNA fragments (1 μg) digested with 50-fold excess enzyme can be ligated by T4 DNA ligase (400 U) at 16℃ for 16 hr. Of the ligation products, > 95% can be re-cut.

Blue/white screening: To test the integrity of DNA ends, a plasmid, pSKM2 containing a unique site in the lac Z alpha gene is digested with 10-fold excess enzyme, ligated, and introduced into DH5α. The transformed cells are plated on X-gal/IPTG/Amp plates. The number of blue and white colonies formed are measured. Blue colonies indicate that an intactness of the test gene is maintained during the cleavage/ligation process. In contrast, white colonies fail to do so. Fewer than 1% white colonies are formed with enzyme.

Extreme pure (EP): No detectable degradation of ^{32}P-end labeled single-stranded and double-stranded (5'-, 3'-overhang and blunt end) oligonucleotides occurred during incubation with 100 U of enzyme for 4 hr.

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Recognition site

5'···GGCCNNNN↓NGGCC···3'
3'···CCGGN↑NNNNCCGG···5'

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ISO9001 ISO14001 ISO13485

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