enzynomics
www.enzynomics.com
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StyD4 I

Cat.#	Size	Conc.
R112S	200 units	5 units/μl
R112M	400 units	5 units/μl
R112L	1,000 units	5 units/μl

Expire date:

Store at -20℃

Supplied with: 10X EzBuffer IV
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: *Salmonella typhi* D4

Reaction conditions
1X EzBuffer IV, 37℃
1X FastCut Buffer, 37℃

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

1X EzBuffer IV
20 mM Tris-acetate (pH 7.9 @ 25℃)
50 mM potassium acetate
10 mM magnesium acetate
100 μg/ml BSA

Unit definition
One unit is defined as the amount of enzyme required to digest 1 μg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 μl.

Storage
10 mM Tris-HCl (pH 7.4), 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 500 μg/ml BSA, 50% glycerol

Dilution buffer: EzDiluent B
10 mM Tris-HCl (pH 7.4 @ 25℃), 300 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 500 μg/ml BSA, 50% glycerol.

Heat Inactivation
StyD4 I can be inactivated at 65℃ for 20 min.

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

Relative activity in EzBuffers

EzBuffer I:	10%
EzBuffer II:	100%
EzBuffer III:	100%
EzBuffer IV:	100%
FastCut Buffer:	100%

Note
Cleavage is blocked by *dcm* methylation overlapping its recognition sequence. Cleavage of mammalian genomic DNA can be blocked by CpG methylation that partially overlaps its recognition sequence.

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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5' ··· C C N G G ··· 3'
3' ··· G G N C C ··· 5'

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