EURx® MOLECULAR BIOLOGY PRODUCTS

HpySP4III

5'-A C N G T-3'
3'-T G N C A-5'

Cat. No.	size
E2270-01	250 units
E2270-02	1250 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: Tsp4CI

Isoschizomers: HpyCH4III, TaaI, Bst4CI

Source: Recombinant. Purified from *E. coli* strain carrying the cloned HpySP4III from *Helicobacter pylori sp. P4*.

Package Contents:

- **HpySP4III**
- **10 x ONE Buffer**
- **BSA [100x]**
  Added as separate component to prevent reaction buffer precipitation.

Storage Conditions: Store at -20°C.

Double Digestion – Buffer Compatibility:

ONE Buffer is compatible with most EURx restriction enzymes.

Restriction Enzyme 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.

DNA Methylation:

No Inhibition: dam, dcm, CpG

Notes:

1. Overdigestion more than 4 hours with over 5 u per µg DNA of enzyme is not recommended.
2. As a result of HpySP4III DNA digestion single 3' extensions are produced, which are difficult to ligate.

Standard Reaction Protocol (for 50 µl volume):

Mix the following reaction components:

1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)

5 µl 10 x ONE Buffer

0.5 µl BSA [100x]

1-2 U HpySP4III (use 1 U per µg DNA, < 10% React. Volume!)

Tips: 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.

add sterile H_2O to 50 µl final volume

Incubate for 1 h at 37°C

To obtain complete digestion of high molecular weight DNA, (e.g. plant genomic DNA), add excess amounts of enzyme and prolong the incubation time.

Stop reaction by alternatively

(a) Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*

(b) Heat Inactivation

20 min at 65°C *or*

(c) Spin Column DNA Purification

(e.g. EURx PCR/DNA Clean-Up Kit, Cat.No. E3520) *or*

(d) Gel Electrophoresis and Single Band Excision

(e.g. EURx Agarose-Out DNA Kit, Cat.No. E3540) *or*

(e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Unit Definition:

One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA in 1 hr. Total reaction volume is 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x ONE Buffer

To be supplemented with 100 µg/ml bovine serum albumin.

Storage Buffer:

20 mM Tris-HCl (pH 7.5 at 22°C), 100 mM NaCl, 1 mM dithiothreiol, 0.1 mM EDTA, 200 µg/ml bovine serum albumin and 50% (v/v) glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as nonspecific single- and double-stranded DNase activities.

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