# PspXI

NEW ENGLAND BioLabs Inc.
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R0656S 0011209140 91

## R0656S

NEB4 37° Yes

**200 units** **5,000 U/ml** **Lot: 0011209**
**RECOMBINANT** **Store at –20°C** **Exp: 9/14**

**Recognition Site:**

5′. . . V C▼T C G A G B . . . 3′
3′. . . B G A G C T▲C V . . . 5′

**Single Letter Code:** B = C or G or T,
V = A or C or G

**Source:** An *E. coli* strain that carries the cloned PspXI gene from *Pseudomanas* species A1–1 (S.K. Degtyarev)

**New Reaction Buffer**

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 @25°C), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X NEBuffer 4.

**Reaction Conditions:** 1X NEBuffer 4. Incubate at 37°C.

**1X NEBuffer 4:**
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM dithiothreitol
pH 7.9 @ 25°C

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

**Diluent Compatibility:** Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

## Quality Control Assays

**Ligation:** After a 10-fold overdigestion with PspXI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of DNA and 25 units of enzyme incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by gel electrophoresis.

**Exonuclease Activity:** Incubation of a 50 µl reaction containing 50 units of PspXI with 1 µg of a mixture of single and double-stranded [$^3$H] *E. coli* DNA (200,000 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

## Enzyme Properties

**Activity in NEBuffers:**

| | |
|---|---|
| NEBuffer 1 | 0% |
| NEBuffer 2 | **100%** |
| NEBuffer 3 | 10% |
| NEBuffer 4 | **100**% |

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

**Survival in a Reaction:** A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

**Heat Inactivation:** 80°C for 20 minutes.

**Note:** Incubation at 50°C results in 30% activity.

**Single Letter Code**
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M = A or C
R = A or G
S = C or G
W = A or T
Y = C or T
B = C or G or T
D = A or G or T
H = A or C or T
V = A or C or G
N = A or C or G or T

CERTIFICATE OF ANALYSIS

PspXI

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