



BSA 37° 🐝 🥯

info@neb.com www.neb.com 🗙 RX 🕐 NEB 3 **R0140S** 

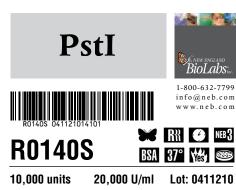
20,000 U/ml Lot: 0411210 10.000 units RECOMBINANT Store at -20°C Exp: 10/14

#### **Recognition Site:**

5′... C T G C A<sup>T</sup>G ... 3′ 3′... GACGTC...5′

Source: An E. coli strain that carries the cloned Pstl gene from Providencia stuartii 164 (ATCC 49762)

Supplied in: 200 mM NaCl, 10 mM Tris-HCl (pH 7.4). 0.1 mM EDTA. 1 mM DTT. 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.



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**Reagents Supplied with Enzyme:** 10X NEBuffer 3, 100X BSA.

Reaction Conditions: 1X NEBuffer 3, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 3: 100 mM NaCl 50 mM Tris-HCI 10 mM MaCl. 1 mM DTT pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 ug of  $\lambda$  DNA in 1 hour at 37°C in a total reaction volume of 50 ul.

Diluent Compatibility: Diluent Buffer C 250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT. 0.15% Triton X-100. 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C).

## **Quality Control Assays**

Ligation: After 100-fold overdigestion with Pstl. > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of  $1-2 \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

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16-Hour Incubation: A 50 µl reaction containing 1 ug of DNA and 400 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 400 units of enzyme with 1 µg sonicated <sup>3</sup>H DNA (10<sup>5</sup> cpm/ µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within  $lacZ^{\alpha}$  gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of  $\beta$ -galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties Activity in NEBuffers:

NEBuffer 1 75% NEBuffer 2 75% NEBuffer 3 100% NEBuffer 4 50%

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 0.50 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 1 unit. pBR322 = 1 unit. LITMUS = 1 unit.

Note: Not sensitive to *dam, dcm* or mammalian CpG methylation.

E = Time-Saver™ Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

# **Enzyme Properties**

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NEBuffer 3	100%

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