PmeI

1-800-632-7799
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www.neb.com

R0560S 016121214141

R0560S

500 units **10,000 U/ml** **Lot: 0161212**

RECOMBINANT **Store at –20°C** **Exp: 12/14**

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned PmeI gene from *Pseudomonas mendocina* (B. Zhou)

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

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. 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 in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 10-fold overdigestion with PmeI, > 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 10 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 100 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released 0.05% radioactivity.

Endonuclease Activity: Incubation of 16 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in a 50 µl reaction buffer resulted in 5% conversion to RF II.

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 β-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	0%
NEBuffer 2	50%
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.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 1.00 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 20 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pNEB193 = 4 units.

Notes: PmeI is an octanucleotide recognizing restriction endonuclease.

Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation.

pNEB193 Vector (NEB #N3051), a cloning vector which contains single sites for NEB's unique 8-base cutters AscI, PacI and PmeI is available.

Four units of PmeI are required for complete digestion of 1 µg of pNEB193.

For extended digests, incubation at 25°C increases enzyme stability.

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

U.S. Patent No. 5,196,330 and 5,945,228

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