PpuMI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R0506S 032120714071

R0506S

NEB4 37° dcm No

500 units	**5,000 U/ml**	**Lot: 0321207**
RECOMBINANT	**Store at –20°C**	**Exp: 7/14**

Recognition Site:

5′. . . R G▼G W C C Y . . . 3′
3′. . . Y C C W G▲G R . . . 5′

Single Letter Code: R = A or G, W = A or T, Y = C or T

Source: An *E. coli* strain that carries the cloned PpuMI gene from *Pseudomonas putida* (R. Morgan)

Supplied in: 50 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.

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 dithiothreitol , 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 20-fold overdigestion with PpuMI, > 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 300 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 300 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 75 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in 5% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	25%
NEBuffer 3	0%
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: No

Note: Blocked by overlapping *dcm* methylation.

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

CERTIFICATE OF ANALYSIS

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