

PfI



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



R0595S 007120714071

R0595S



2,000 units Lot: 0071207 Exp: 7/14
RECOMBINANT 10,000 U/ml Store at -20°C

Recognition Site:

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

Source: An *E. coli* strain that carries the cloned PfI gene from *Pseudomonas fluorescens* F (C. Polisson)

More Units, Now Recombinant

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 pBC4 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 PfI, approximately 25% 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 20 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 [³H] DNA (10⁵ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.2% radioactivity.

Endonuclease Activity: Incubation of 10 units of enzyme with 1 µg φX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 5% detectable conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	0%
NEBuffer 2	25%
NEBuffer 3	25%
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: 100 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 1 hour: pBR322 = 2 units.

(See other side)

CERTIFICATE OF ANALYSIS

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CERTIFICATE OF ANALYSIS

Notes: PfiFI has 20-fold less star activity than its isoschizomer TthIII under standard reaction conditions.

PfiFI produces DNA fragments that have a single-base 5' extension which are more difficult to ligate than blunt-ended fragments.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation

Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity.


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

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