PhoI

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

R0705S 002120814081

R0705S

200 units **2,000 U/ml** **Lot: 0021208**
RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′...GG▼CC...3′
3′...CC▲GG...5′

Source: An *E. coli* strain that carries the cloned PhoI gene from *Pyrococcos horikoshii* OT3 (Y. Kawarabayasi)

Supplied in: 50 mM KCl, 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 3

Reaction Conditions: 1X NEBuffer 3.
Incubate at 75°C.

1X NEBuffer 3:
100 mM NaCl
50 mM Tris-HCl
10 mM $MgCl_2$
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 75°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 2-fold overdigestion with PhoI, approximately 50% 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, approximately 50% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ 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 20 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 < 0.1% radioactivity.

*This quality control was performed at 37°C to detect any *E. coli* contaminants which are not reactive at 75°C.

Enzyme Properties

Activity in NEBuffers:

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

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: PhoI is a highly thermostable restriction enzyme that can survive temperatures as high as 95°C.

Impaired by some combinations of overlapping *dcm* methylation.

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

Incubation at 37°C results in no activity.

Companion Products:

dam⁻/dcm⁻ Competent *E. coli*

#C2925H	20 transformation reactions
#C2925I	24 transformation reactions

CERTIFICATE OF ANALYSIS

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