PhoI

**Reagents Supplied with Enzyme:**
10X NEBuffer 3

**Reaction Conditions:**
1X NEBuffer 3:
- 100 mM NaCl
- 50 mM Tris-HCl
- 10 mM MgCl₂
- 1 mM dithiothreitol
- pH 7.9 @ 25°C

**Incubate at 75°C.**

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 [³H] DNA (10⁵ 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**

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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

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**Recognition Site:**
- 5’…G G C C C…3’
- 3’…C G G G G…5’

**Source:** An *E. coli* strain that carries the cloned PhoI gene from *Pyrococcus 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.

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