XhoI

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

R0146S 058120614061

R0146S

5,000 units **20,000 U/ml** **Lot: 0581206**
RECOMBINANT **Store at –20°C** **Exp: 6/14**

Recognition Site:

5′...C▼TCGAG...3′
3′...GAGCT▲C...5′

Source: An *E. coli* strain that carries the cloned XhoI gene from *Xanthomonas holcicola* (ATCC 13461)

New Reaction Buffer

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 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 DTT
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) fragments 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 XhoI, > 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 200 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 400 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.

Endonuclease Activity: Incubation of 100 units of enzyme with 1 µg pBR322 DNA for 4 hours at 37°C in 50 µl reaction buffer gave < 10% conversion to RF II.

Blue/White Screening Assay: An appropriate vector is digested at a unique site within the *lacZ*$^{\alpha}$ gene with a 10-fold excess of enzyme. The vector DNA is then ligated, transformed, and plated onto 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, removal of even a single base gives rise to a white colony. Enzyme preparations must produce fewer than 3% white colonies to be Blue/White certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	75%
NEBuffer 2	**100%**
NEBuffer 3	100%
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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CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 0.13 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 65°C for 20 minutes.

Notes: XhoI is an isoschizmoer of PaeR7I.

Cleavage of mammalian genomic DNA is impaired by CpG methylation.

Companion Products Sold Separately:

XhoI RE-Mix™
#R5146S 200 reactions

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

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