SpeI



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





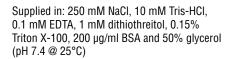
500 units 10.000 U/ml Lot: 0321211 RECOMBINANT Store at -20°C Exp: 11/14

Recognition Site:

5'... A C T A G T ... 3' 3′... T G A T C, A ... 5′

Source: An *E. coli* strain that carries the cloned Spel gene from Sphaerotilus species (ATCC 13923)

New Reaction Buffer



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 Adenovirus-2 DNA in 1 hour at 37°C in a total reaction volume of 50 ul.

Diluent Compatibility: Diluent Buffer C: 250 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 ug/ ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 20-fold overdigestion with Spel. > 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 Adenovirus-2 DNA and 50 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 < 0.1% radioactivity.

Endonuclease Activity: Incubation of 50 units of enzyme with 1 ug ϕ X174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 20% 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% 25% NEBuffer 3 100% NEBuffer 4

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 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: 80°C for 20 minutes.

(See other side)

CERTIFICATE OF ANALYSIS

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Note: Cleaves to leave a 5´ CTAG extension which can be efficiently ligated to DNA fragments generated by AvrII, Nhel, or Xbal.

Not sensitive to $\it dam, \it dcm$ or mammalian CpG methylation.

= Time-Saver™ Qualified

U.S. Patent No. 5,945,326

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