SpeI

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

R0133S 032121114111

R0133S

500 units **10,000 U/ml** **Lot: 0321211**
RECOMBINANT **Store at –20°C** **Exp: 11/14**

Recognition Site:

5′. . . A▼CTAGT . . . 3′
3′. . . TGATC▲A . . . 5′

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

New Reaction Buffer

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

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 µl.

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 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 20-fold overdigestion with SpeI, > 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 ^{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 50 units of enzyme with 1 µg ϕX174 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%**
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 0.25 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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NEBuffer 3 25%
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(See other side)
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Note: Cleaves to leave a 5´ CTAG extension which can be efficiently ligated to DNA fragments generated by AvrII, NheI, or XbaI.

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

= Time-Saver™ Qualified

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

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