NspI

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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R0602S 010120514051

R0602S

NEB2 BSA 37° Yes

250 units **10,000 U/ml** **Lot: 0101205**

RECOMBINANT **Store at –20°C** **Exp: 5/14**

Recognition Site:

5′...RCATG▼Y...3′
3′...Y▲GTACR...5′

Single Letter Code: R = A or G, Y = C or T

Source: An *E.coli* strain that carries the cloned NspI gene from *Nostoc* species C (ATCC 29411)

Higher Concentration

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2, 100X BSA.

Reaction Conditions: 1X NEBuffer 2, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 2:
50 mM NaCl
10 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 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 50-fold overdigestion with NspI, > 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 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 200 units of enzyme with 1 µg sonicated ^3H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 20 units of enzyme with 1 µg ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	100%
NEBuffer 2	**100%**
NEBuffer 3	0%
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: 65°C for 20 minutes.

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

Nsp I dilutions must be supplemented with 0.15% Triton X-100.

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

U.S. Patent No. 6,027,929

CERTIFICATE OF ANALYSIS

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