Nuclease BAL-31

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

M0213S 040130315031

M0213S

Yes

50 units **Lot: 0401303** **Exp: 3/15**

1,000 U/ml **Store at –20°C**

Description: BAL-31 exonuclease degrades both 3´ and 5´ termini of duplex DNA without generating internal scissions. The enzyme is also a highly specific single-stranded endonuclease which cleaves at nicks, gaps and single-stranded regions of duplex DNA and RNA (1,2).

Source: Purified from the culture medium of *Alteromonas espejiana* BAL-31. Contains a mixture of "fast" and "slow" species of the enzyme (3).

Applications:

- Progressive shortening of double-stranded DNA fragments at both termini (4)
- Restriction site mapping (2).

Supplied in: 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1.5 mM $CaCl_2$,1.5 mM $MgCl_2$, 0.25 mM EDTA, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
2X Nuclease BAL-31 Reaction Buffer.

Reaction Conditions: 1X Nuclease BAL-31 Reaction Buffer. **Incubate at 30°C.**

1X Nuclease BAL-31 Reaction Buffer:
600 mM NaCl
12 mM $CaCl_2$
12 mM $MgCl_2$
20 mM Tris-HCl
1 mM EDTA
pH 8.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to remove 200 base pairs from each end of linearized double-stranded ϕX174 DNA (40 µg/ml) in 50 µl of 1X Nuclease BAL-31 Reaction Buffer in 10 minutes at 30°C.

Heat Inactivation: Heat inactivated by incubation at 65°C for 10 minutes in the presence of 20 mM EGTA, a specific chelator of the essential cofactor Ca^{2+}. This treatment does not affect the Mg^{2+} concentration.

Quality Control Assays

Ligation Activity: Incubation of 50 µl reaction buffer containing 30 units of enzyme and 25 µg HaeIII fragments of ϕX174 RF I DNA for 5 minutes at 30°C allowed the ligation of approximately 75% of the fragments during subsequent incubation with T4 DNA Ligase (5´ termini concentration-20 µM).

Double-Stranded Endonuclease Activity: Incubation of 60 units of Nuclease BAL-31 with 65 µg λ DNA for 10 minutes at 30°C in 100 µl reaction buffer (rendering 50% of the DNA acid-soluble) resulted in no detectable endonuclease activity. This is judged by the integrity of the internal λ DNA fragments produced by subsequent digestion with Hind III endonuclease.

Notes On Use: Duplex products of the exonuclease are a mixture of blunt and staggered ends. This mixture can be cloned directly, although maximal ligation efficiency requires repairing the staggered ends with a suitable DNA polymerase.

If necessary, the enzyme may be diluted in reaction buffer prior to use.

Activity is linear with enzyme concentration.

References:

1. Gray, H. B. et al. (1975) *Nucleic Acids Res.* 2, 1459–1492.
2. Legerski, R. J. et al. (1978) *Nucleic Acids Res.* 5, 1445–1463.
3. Wei, C. -F. et al. (1983) *J. Biol. Chem.* 258, 13506–13512.
4. Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 5.73–5.75). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

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