**Nuclease BAL-31**

**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 CaCl₂, 1.5 mM MgCl₂, 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:
- 600 mM NaCl
- 12 mM CaCl₂
- 12 mM MgCl₂
- 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 Ca²⁺. This treatment does not affect the Mg²⁺ concentration.

### Quality Control Assays

**Ligation 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.

**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:**