Exonuclease T

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M0265S 003130315031

M0265S

250 units **5,000 U/ml** **Lot: 0031303**

RECOMBINANT **Store at –20°C Exp: 3/15**

Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA (1,2) or DNA (3,4) specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exonuclease T can be used to generate blunt ends from RNA (5) or DNA molecules that have 3´ extensions (2).

Source: Exonuclease T is overexpressed and purified as a C-terminal fusion to maltose-binding protein (MBP). MBP is removed from Exonuclease T by Factor Xa cleavage and Exonuclease T is then purified away from Factor Xa and MBP. Exonuclease T cleaved from MBP has an additional amino acid on the N-terminus and a Phe instead of a Met (i.e. Glu-Phe-Exo T instead of Met-Exo T).

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

Reagents Supplied with Enzyme:
10X NEBuffer 4

Reaction Conditions: 1X NEBuffer 4.
Incubate at 25°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 produce 0.1 nmol of TCA soluble DNA from 1 nmol of [^{3}H]-labeled polythymidine in 30 minutes at 25°C in a total reaction volume of 100 µl.

Unit Assay Conditions: 1X NEBuffer 4, 1 nmol [^{3}H]-labeled polythymidine DNA and enzyme.

Heat Inactivation: 65°C for 20 minutes.

Notes On Use: Exo T has different activity on RNA vs. DNA. For RNA, 1 unit of Exo T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions as measured by gel electrophoresis.

Quality Control Assays

5´→ 3´ ss and ds Exonuclease Activity: No detectable 5´→ 3´ nuclease activity was observed when 10 units of Exonuclease T was incubated with substrates containing either 5´ extensions or blunt ends.

Endonuclease Activity: Incubation of 10 units of Exonuclease T with 1 µg ϕX174 RF I DNA for 4 hours at 25°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.

Quality Assurance: Free of detectable endonucleases and exonucleases.

References:

1. Deutscher, M. P., Marlor, C. W. and Zaniewski, R. (1984) *Proc. Natl. Acad. Sci. USA* 81, 4290–4293.
2. Deutscher, M. P. and Marlor, C. W. (1985) *J. Biol. Chem.* 260, 7067–7071.
3. Viswanathan, M., Dower, K. D. and Lovett, S. T. (1998) *J. Biol. Chem.* 273, 35126–35131.
4. Zuo, Y. and Deutscher, M. P. (1999) *Nucleic Acid Res.* 27, 4077–4082.
5. Zeng, Y. and Cullen, B. R. (2004) *Nucleic Acid Res.* 32, 4776–4780.

CERTIFICATE OF ANALYSIS

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5´→ 3´ ss and ds Exonuclease Activity: No detectable 5´→ 3´ nuclease activity was observed when 10 units of Exonuclease T was incubated with substrates containing either 5´ extensions or blunt ends.

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