Exonuclease T

**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).

**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 [3H]-labeled polythymidine in 30 minutes at 25°C in a total reaction volume of 100 µl.

**Unit Assay Conditions:** 1X NEBuffer 4, 1 nmol [3H]-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.

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**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:**


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**M0265S 0031505**

**250 units 5,000 U/ml Lot: 0031505**

**RECOMBINANT Store at –20°C Exp: 5/17**

**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).

**Reagents Supplied with Enzyme:**

- 10X NEBuffer 4

**Reaction Conditions:**

1X NEBuffer 4:

- 50 mM potassium acetate
- 20 mM Tris-acetate
- 10 mM magnesium acetate
- 1 mM dithiothreitol
- pH 7.9 @ 25°C

**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.

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**References:**

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