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**

1. **Endonuclease Activity:** Incubation of 10 units of Exonuclease T with 1 µg X174 RF I DNA for 4 hours at 25°C in 50 µl reaction buffer resulted in < 10% conversion to RF II.
2. **Quality Assurance:** Free of detectable endonucleases and exonucleases.

**References:**

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