

## T5 Exonuclease, *E. coli*

Cat. No. T5E4111K

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## 1. Introduction

T5 Exonuclease, (T5 Exo) derived from *E. coli*, is a highly efficient 5'→3' exonuclease for either single-strand or duplex DNA. It has a tightly associated single-strand specific endonuclease activity when used in the presence of 1-10 mM Mg<sup>2+</sup>. This activity may be selectively suppressed by using low concentrations of magnesium ions (<1 mM), allowing nicked, double-stranded circular DNA to be "gapped" to a single-strand circular species.<sup>1</sup> The mode of action of T5 Exo *in vivo* may be analogous to that of the 5'→3' exonuclease activity of *E. coli* DNA polymerase I.<sup>2</sup> In the absence of divalent metal cofactors, T5 Exo is able to bind DNA with a single-stranded arm adjacent to a duplex DNA region.<sup>3</sup>

T5 Exo is available in a 1,000 Unit size at a concentration of 10 Units/μl. The enzyme is supplied with a 10X Reaction Buffer.

## Applications

- Plasmid mutagenesis methods.
- Oligonucleotide site-directed mutagenesis.
- Generation of plasmid-sequencing templates.
- Removal of denatured DNA from alkaline-based plasmid purification procedures for improved cloning.<sup>4</sup>

## 2. Product Specifications

**Storage:** Store only at -20°C in a freezer without a defrost cycle.

**Storage Buffer:** T5 Exo is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1.0 mM dithiothreitol (DTT), and 0.1% Triton® X-100.

**Unit Definition:** One unit of T5 Exo results in the acid-solubilization of 1 nmol of nucleotides from double-stranded calf thymus DNA in 30 minutes at 37°C.

**10X T5 Exonuclease Reaction Buffer:** 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT.

**Quality Control:** T5 Exo is function-tested in a reaction containing 33 mM Tris-acetate (pH 7.5), 66 mM potassium acetate, 10 mM magnesium acetate, 0.5 mM DTT, 20 μg of double-stranded calf thymus DNA, and varying amounts of T5 Exo.

**Contaminating Activity Assays:** T5 Exo is free of detectable RNase and DNA endonuclease activities.

### 3. Related Products

The following products are also available:

- Exonuclease I, *E. coli*
- Exonuclease III, *E. coli*
- Exonuclease VII, *E. coli*
- Lambda Exonuclease
- Rec BCD Nuclease
- Rec J Exonuclease, *E. coli*
- Terminator™ 5'-Phosphate-Dependent Exonuclease
- Mung Bean Nuclease
- OmniCleave™ Endonuclease

### 4. References

1. Sayers, J.R. and Eckstein, F. (1991) *Nucl. Acids Res.* **19**, 4127.
2. Sayers, J.R. and Eckstein, F. (1990) *J. Biol. Chem.* **265**, 18311.
3. Garforth, S.J. and Sayers, J.R. (1997) *Nucl. Acids Res.* **25**, 3801.
4. Sayers, J.R. *et al.*, (1996) *Anal. Biochem.* **241**, 186.

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