Description: Exonuclease V, a RecBCD complex from *E. coli* has several different enzyme activities, including an ATP-dependent single-stranded DNA endonuclease activity, ss- and ds- DNA exonuclease activity. The hydrolysis in each case is bi-directional (from both the 3’ and 5’ ends) and processive, producing oligonucleotides (1,2,3). All Exonuclease V activities have divalent cation requirements. Mg²⁺ is required for the exonuclease activity, while Ca²⁺ inhibits the exonuclease activity and allows double-stranded DNA unwinding (helicase activity) without hydrolysis (4,5).

**Source:** An *E. coli* strain containing plasmids for expressing the three subunits of *E. coli* Exonuclease V: RecB, RecC and RecD.

Supplied in: 100 mM NaCl, 50 mM Tris-HCl (pH 7.5 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

**Reagents Supplied with Enzyme:**
- 10X NEBuffer 4, 10 mM ATP
- 3 µl NEBuffer 4
- 3 µl 10 mM ATP
- y µl H₂O (up to final volume of 30 µl)

**Unit Assay Conditions:**
1X NEBuffer 4, 1 mM ATP with 0.15 mM sonicated duplex [H]-DNA.

**Unit Assay Definition:**
One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37°C in a total reaction volume of 50 µl.

**Heat Inactivation:** 70°C for 30 minutes.

**Quality Control Assays**

**Endonuclease Activity I:** Incubation of a 50 µl reaction containing 100 units of Exonuclease V with 1 µg of φX174 RF I DNA in NEBuffer 4 and 1 mM ATP for 4 hours at 37°C resulted in < 10% loss in φX174 RF I DNA as determined by agarose gel electrophoresis. **Endonuclease Activity II:** Incubation of a 50 µl reaction containing 50 units of Exonuclease V with 1 µg of φX174 RF II DNA in NEBuffer 4 and 1 mM ATP for 4 hours at 37°C resulted in < 10% loss in φX174 RF II DNA as determined by agarose gel electrophoresis.

**RNase Assay:** Incubation of 10 units of enzyme with 40 ng of 300 bases Fam-labeled RNA at 37°C for 3 hours in a 50 µl reaction buffer results in < 10% of RNA degradation as analyzed by polyacrylamide gel.

**Physical Purity:** Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

**A Typical Exonuclease V Reaction:**
- x µl sample DNA (~ 1 µg)
- 3 µl NEBuffer 4 (10X)
- 3 µl 10 mM ATP
- y µl H₂O (up to final volume of 30 µl)

**Note:** Estimate amount of DNA to be removed by agaurge gel electrophoresis or OD₂₆₀. If > 1 µg scale up all reaction components proportionately.

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