Certificate of Analysis

Part# 9PIM181

Revised 11/20

Exonuclease III:

Part No.	Size (units)
M181A	5,000
M181C	25,000

Exonuclease III 10X Reaction Buffer (E577A): The Exonuclease III 10X Reaction Buffer supplied with this enzyme has a composition of 660mM Tris-HCl (pH 8.0) and 6.6mM $MgCl_2$.

Enzyme Storage Buffer: Exonuclease III is supplied in 20mM Tris-HCl (pH 7.5), 1mM DTT, 100mM KCl and 50% glycerol.

Source: *E. coli* cells expressing a recombinant clone.

Unit Definition: The unit definition for Exonuclease III has been changed. One unit of Exonuclease III is defined as the amount of enzyme required to produce 1nmol of acid-soluble nucleotides from double-stranded DNA in 30 minutes at 37°C. See the unit concentration on the Product Information Label. One unit as defined by these conditions is equivalent to one unit under the previous unit definition for this product.

Storage Temperature: Store at –30°C to –10°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

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Quality Control Assays

Activity Assay

3´-Overhang Protection Assay: Twenty-five units of Exonuclease III are incubated with 1µg of Pst I cut plasmid at 37°C for 4 minutes. The plasmid DNA shows <10% degradation when visualized by ethidium bromide-stained agarose gel electrophoresis.

Contaminant Activity

Endonuclease Assay: To test for endonuclease activity, 1µg of Type I supercoiled plasmid DNA is incubated with 50 units of Exonuclease III in 50mM Tris-HCl (pH 7.6), 10mM $MgCl_2$, 1mM DTT for 1 hour at 37°C. Following incubation, the supercoiled DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible nicking or cutting.

Physical Purity: The purity of Exonuclease III is >90% as judged by SDS-polyacrylamide gels with Coomassie® blue staining.

Signed by:

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Usage Information

1. Description

Exonuclease III (Exo III) has a double-strand specific, nonprocessive 3´→5´ exo-deoxyribonuclease activity; however, 3´-overhangs of ≥4 bases are protected from Exo III activity (1).

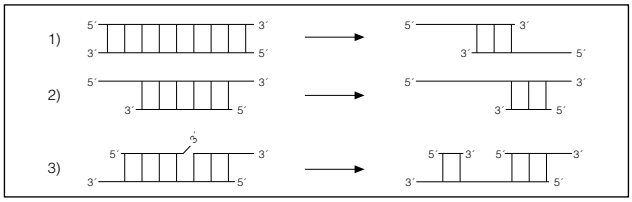

Figure 1. Exonuclease III catalyzes the stepwise removal of mononucleotides starting from a 3´-OH at: 1) blunt ends, 2) recessed ends and 3) nicks. Exonuclease III will also act on 3´-overhangs of less than 4 bases (not shown). Note that the 3´-overhangs shown in 3) are ≥4 bases and therefore not susceptible to Exonuclease III activity.

2. Digestion with Exonuclease III

A. Standard Applications

Exonuclease III catalyzes the stepwise removal of mononucleotides starting from a 3´-OH at nicks, blunt or recessed ends and 3´-overhangs of less than 4 bases, yielding nucleoside 5´-phosphates. Exonuclease III will also degrade from 3´-phosphate ends, due to an intrinsic 3´-phosphatase activity (2). In addition, the enzyme has apurinic endonuclease activity and ribonuclease H activity (2). Exonuclease III is used in conjunction with S1 nuclease for unidirectional deletion of sequences from the termini of DNA fragments (3).

B. Reaction Conditions for Timed, Unidirectional Deletions Using Exo III and S1 Nuclease

Dissolve 5µg of doubly cut plasmid DNA (one restriction enzyme should produce a 4-base, 3´-overhang, which will be protected from Exo III digestion, and the other enzyme should produce a 5´-overhang or blunt end adjacent to the region from which the deletions will proceed) in 60µl 1X Exo III Reaction Buffer (66mM Tris-HCl [pH 8.0], 0.66mM $MgCl_2$). Meanwhile, add 7.5µl of S1 nuclease mix (200µl final volume containing 40mM potassium acetate (pH 4.6), 340mM NaCl, 1.35mM $ZnSO_4$, 6.8% glycerol and 60 units S1 nuclease) to each of 25 x 0.5ml microcentrifuge tubes and leave on ice. Warm the DNA tube to the digestion temperature in a water bath (see Note, Section 2.C). Add 250–500 units of Exo III, mixing as rapidly as possible. At 30-second intervals, transfer 2.5µl samples into the S1 tubes on ice, pipetting briefly to mix. After all the samples have been taken, move the tubes to room temperature for 30 minutes.

Next, add 1µl of S1 stop buffer (0.3M Tris base, 0.05M EDTA), and heat at 70°C for 10 minutes to inactivate the S1 nuclease. Fill in with Klenow fragment to flush the ends. To determine the extent of digestion, remove 2µl samples (about 40ng DNA) from each time point for analysis on a 1% agarose gel.

C. Reaction Conditions for 3´→5´ Double-Ended Deletions

Add 2µg of digested DNA with either blunt ends or 5´-overhangs to a 50µl reaction containing 50mM Tris-HCl (pH 7.5), 5mM $MgCl_2$, 5mM DTT and 50µg/ml BSA. Add 10 units of Exo III and mix. Incubate at 37°C for 1–30 minutes, depending upon the amount of digestion required. Stop the reaction by adding 2µl of 0.5M EDTA or by heating at 75°C for 10 minutes (4).

Note: Unidirectional digestion proceeds at approximately 500 bases/minute at 37°C (Table 1). There is a 20–30 second lag before the reaction begins when incubated at 37°C. The rate of Exo III digestion can vary depending on the incubation temperature (lag times will increase as the temperature is decreased; 5), the DNA template used and the NaCl concentration (see Section 3).

Table 1. Temperature Dependence of Exonuclease III Digestion Rate.

Temperature	Rate of Exonuclease III Digestion
22°C	approximately 60bp/minute
25°C	approximately 100bp/minute
30°C	approximately 200bp/minute
37°C	approximately 500bp/minute
40°C	approximately 600bp/minute

3. Additional Information

Molecular Weight: 28,000Da.

Inactivation: 75°C for 10 minutes or 20mM EDTA.

Optimum pH Range: 7.6-8.5 in Tris-HCl buffer.

Divalent Cations: Exonuclease III is partially active in the absence of added divalent cations. Mg^{2+} or Mn^{2+} is required for optimum activity; both cations are equally effective (6).

Inhibitor: The presence of greater than 50mM NaCl substantially slows the rate of deletion.

4 References

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3. Henikoff, S. (1984) Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene* **28**, 351–59.
4. Ausubel, F.M. *et al.* (1988) Current Protocols in Molecular Biology, John Wiley and Sons, New York.
5. Henikoff, S. (1987) Unidirectional digestion with exonuclease III in DNA sequence analysis. *Meth. Enzymol.* **155**, 156–65.
6. Boyer, P.D. (1981) The Enzymes, Vol. XIV, Part A, 3rd ed., Academic Press, New York.

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