Exonuclease III
Tested User Friendly™
Product Number 70023
Deletion Protocol

Exonuclease III, the major apurinic/apyrimidinic (AP) endonuclease in E. coli, is a 3'-5' exonuclease specific for dsDNA. This enzyme catalyzes the step-wise removal of 5'-mononucleotides from the 3'-ends of dsDNA but not from ssDNA. Exonuclease III is active on dsDNA with blunt ends, 5'-overhangs, and nicks, but not on protruding 3'-ends 4 bases or longer. These properties are utilized to facilitate strand-specific labeling, unidirectional deletions, and selective strand degradation. Exonuclease III also has several other activities including: digestion of the RNA strand of an RNA-DNA heteroduplex, strand-cleavage on the 5' side of AP sites in both dsDNA and ssDNA, removal of 3'-phosphates from dsDNA, and an increase in MutY turnover.

10X Exonuclease III Reaction Buffer (PN 70096, included): 660mM Tris-HCl (pH 8.0), 66mM MgCl₂, 50mM DTT

Deletion Protocol:
Exonuclease III has been functionally tested with S1 Nuclease (PN 70019) in the following uni-directional deletion protocol:

1. Digest 5-10 µg of DNA with two restriction enzymes:
   a) one producing a 4 base 3'-overhang (to protect this end from deletion)
   b) one producing a 5'-overhang or blunt end adjacent to the insert from which deletions are to proceed

2. Extract with 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1). Precipitate DNA by adding 0.1 volume of 2M NaCl and 2 volumes of ethanol. Freeze for 10 min at -20°C. Centrifuge at 10,000 x g for 5 min, rinse pellet with 70% ethanol, decant and dry pellet.

3. Dissolve DNA in 60 µl 1X Exonuclease III buffer.

4. Prepare twenty-five (25) 0.5 ml microcentrifuge tubes, one for each time point, with 7.5 µl S1 mix in each tube and place on ice.

7.4X S1 Buffer:
0.3M Potassium Acetate (pH 4.6), 2.5M NaCl, 10mM ZnSO₄

S1 Mix:
172 µl ddH₂O, 27 µl 7.4X S1 Buffer, 60 units S1 Nuclease

5. Warm the DNA tube to digestion temperature (37°C). Add 250-500 units Exonuclease III and immediately mix thoroughly. Remove 2.5 µl aliquots at 30 sec intervals into separate S1 tubes on ice. After all samples are taken, incubate tubes at room temperature for 30 min.

6. Add 1 µl S1 Stop Buffer and incubate at 70°C for 10 min to heat-inactivate the enzyme.

S1 Stop Buffer:
0.3M Tris (pH 8.0), 0.05M EDTA

7. Remove 2 µl from each time point for a 1% agarose gel to determine the extent of the deletions.