Thermo SCIENTIFIC

**PRODUCT INFORMATION**

# Exonuclease III (Exo III)

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**#_**

**Lot: _** **Expiry Date: _**

| Components | #EN0191 |
|---|---|
| Exonuclease III, 200 U/µL | 4000 U |
| 10X Reaction Buffer | 0.2 mL |

**Store at -20 °C**

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**For Research Use Only.** Not for use in diagnostic procedures.

## Description

Exonuclease III (Exo III) exhibits four different catalytic activities (1):

- 3'→5' exodeoxyribonuclease activity specific for double-stranded DNA:
  Exo III degrades dsDNA from blunt ends, 5'-overhangs or nicks, releasing 5'-mononucleotides from the 3'-ends of DNA strands and producing stretches of single-stranded DNA. It is not active on 3'-overhang ends of DNA that are at least four bases long and do not carry a 3'-terminal C-residue (2); on single-stranded DNA, or on phosphorothioate-linked nucleotides.
- 3'-phosphatase activity:
  Exo III removes the 3'-terminal phosphate and generates a 3'-OH group.
- RNase H activity:
  Exo III exonucleolytically degrades the RNA strand in DNA-RNA hybrids.
- Apurinic/apyrimidinic- endonuclease activity:
  Exo III cleaves phosphodiester bonds at apurinic or apyrimidinic sites to produce 5'-termini that are base-free deoxyribose 5'-phosphate residues.

## Applications

- Creation of unidirectional deletions in DNA fragments in conjunction with S1 Nuclease (2, 3), *see* protocol on back page.
- Generation of a single-stranded template for dideoxy sequencing of DNA (4).
- Site-directed mutagenesis (5).
- Cloning of PCR products (6).
- Preparation of strand-specific probes.

## Source

*E.coli* cells carrying a cloned *E.coli xth* gene.

## Molecular Weight

31 kDa monomer.

## Definition of Activity Unit

One unit of the enzyme catalyzes the release of 1 nmol of acid soluble reaction products from *E.coli* [$^3$H]-DNA in 30 min at 37 °C.

Enzyme activity is assayed in the following mixture: 50 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 1 mM DTT and 0.05 mM sonicated *E.coli* [$^3$H]-DNA.

## Storage Buffer

The enzyme is supplied in: 50 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM DTT and 50% (v/v) glycerol.

## 10X Reaction Buffer

660 mM Tris-HCl (pH 8.0 at 30 °C), 6.6 mM $MgCl_2$.

## Inhibition and Inactivation

- Inhibitors: metal chelators, p-chloromercuri benzoate (50-90% inhibitory at 0.1 mM) (7).
- Inactivated by heating at 70 °C for 10 min.

## Note

- The rate of DNA digestion by Exo III depends upon temperature, salt concentration and the molar ratio of DNA to enzyme in a reaction mixture (4, 8). Optimal reaction conditions should be determined experimentally.
- Activity in Thermo Scientific REase Buffers, % (in comparison to activity in assay buffer)

| Buffers | Activity, % |
|---|---|
| Thermo Scientific FastDigest, FastDigest™ Green, O, R, 2X Thermo Scientific Tango, BamHI, EcoRI | 0-25 |
| G, 1X Tango™ | 25-50 |
| B, Ecl136II, KpnI, PacI, SacI | 100 |

# CERTIFICATE OF ANALYSIS

## Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with Exonuclease III.

**Quality authorized by:**  Jurgita Zilinskiene

*(continued on back page)*

## **Protocol for Generation of Unidirectional Deletions in DNA Fragments** (for 25 time points)

1. Digest 5-10 µg of DNA with a pair of restriction enzymes; one which generates a blunt or 5'-overhanging end adjacent to the target sequence, and another which produces a resistant 4 base 3'-overhang close to the priming site.
2. Extract the reaction mixture with 1 volume of phenol:chloroform (1:1) and then with 1 volume of chloroform:isoamyl alcohol (24:1). Precipitate DNA by adding 0.1 volume of NaCl/glycogen solution (1.1 M NaCl, 0.25 mg/mL glycogen) and 2 volumes of 95% ethanol. Mix and centrifuge at 10,000 rpm for 10 minutes. Wash the pellet with 1 mL of 70% ethanol. Dry the pellet.
3. Dissolve DNA in 50 µL of 1X Exo III reaction buffer.
4. Prepare mixture (S1 Nuclease mix) by adding the following:

| | |
|---|---|
| **5X Reaction Buffer for S1 Nuclease*** | 40 µL |
| **S1 Nuclease** (#EN0321) | 0.5 µL (50 U) |
| **Water, nuclease-free** (#R0581) | to 200 µL |

* 200 mM sodium acetate (pH 4.5 at 25 °C), 1.5 M NaCl and 10 mM $ZnSO_4$)

5. Add 7.5 µL aliquots of the S1 Nuclease mix into each of 25 numbered tubes, place on ice.
6. Select incubation temperature and time as follows:

| **Temperature, °C** | 25 | 30 | 37 | 45 |
|---|---|---|---|---|
| **Digestion rate, bp/min** | 80 | 210 | 420 | 600 |

Mononucleotides are released at base-dependent rates in the order: C>A=T>G. DNA termini of different are degraded at different rates, so these guidelines should only be used as an approximation.

7. Warm the DNA solution to the digestion temperature. Add 500 units Exo III and mix rapidly. Remove 2 µL aliquots at the chosen time intervals and add to the tubes containing the cold S1 Nuclease mix.
8. After all samples have been taken, move the tubes to room temperature for 30 minutes. Add 1 µL S1 "STOP" solution (300 mM Tris base, 50 mM EDTA) and heat at 70 °C for 10 minutes to inactivate S1 Nuclease.
9. Use 7 µL of each sample for gel electrophoresis.

## References


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