For research use only

ISO9001

Exonuclease III (*E.coli*)

Product	Quantity	Cat. No.	Remarks
Exonuclease III (*E.coli*)	10,000 uinit	EBT-3025	100 unit/㎕

Description

Exonuclease III (*E.coli*) catalyzes the stepwise removal of mononucleotides starting from a 3´-OH at nicks, blunt ends, recessed ends and 3´-overhangs of less than 4 bases. Exonuclease III is used in conjunction with S1 nuclease for unidirectional deletion of sequences from the termini of DNA fragments to construct a series of deletion mutants. Exonuclease III is purified from a recombinant *E.coli* strain.

Concentration & Storage Condition

100 unit/μl. Store at -20℃.

Storage Buffer

20 mM Tris-HCl, pH 7.5, 1 mM DTT, 100 mM KCl, 50% glycerol .

10x Reaction Buffer

660 mM Tris-HCl, pH 8.0, 6.6 mM $MgCl_2$. .

Unit Definition

One unit is defined as the amount of enzyme required to produce 1 nmole of acid-soluble nucleotides from double-stranded DNA within 30 min at 37°C.

QC Tests

Activity, exo and endonuclease activity test, SDS-PAGE purity, performance tests.

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Usage Information

1. Timed, Unidirectional Deletions Using Exo III and S1 Nuclease

1) Dissolve 5μg of double cut 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.
2) Add 7.5μl of S1 nuclease mix (200μl final volume containing 40 mM potassium acetate (pH 4.6), 340 mM NaCl, 1.35 mM $ZnSO_4$, 6.8% glycerol and 60 units S1 nuclease) to each of 25 x 0.5 ml microcentrifuge tubes and leave on ice.
3) Warm the DNA tube to the digestion temperature in a water bath.
4) Add 250–500 units of Exo III, mixing as rapidly as possible.
   At 30-second intervals, transfer 2.5 μl 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 min.
5) Add 1μl of S1 stop buffer (0.3 M Tris base, 0.05 M EDTA),
6) Heat at 70°C for 10 min to inactivate the S1 nuclease.
7) Fill in with Klenow fragment to flush the ends.
8) To determine the extent of digestion, remove 2 μl samples (about 40 ng DNA) from each time point for analysis on a 1% agarose gel.

2. 3´→5´ Double-Ended Deletions

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

Note: Unidirectional digestion proceeds at approximately 500 bases/min at 37°C.
There is a 20–30 sec 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), the DNA template used and the NaCl concentration.

3. Temperature Dependence of Exonuclease III Digestion Rate.

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