Exonuclease III (*E. coli*)

Catalog: RK20533
Size: 2,500 U / 5,000 U / 25,000 U
Concentration: 100,000 U/ml
Components:

Exonuclease III (*E.coli*)	RM20521
10X ABuffer A	RM20125

Product Description

Exonuclease III (*E. coli*) catalyzes the stepwise removal of mononucleotides from 3´-hydroxyl termini of duplex DNA. A limited number of nucleotides are removed during each binding event, resulting in coordinated progressive deletions within the population of DNA molecules.

The preferred substrates are blunt or recessed 3´-termini, although the enzyme also acts at nicks in duplex DNA to produce single-strand gaps. 3´-protruding termini are resistant to cleavage; the degree of resistance depends on the length of the extension, with extensions 4 bases or longer being essentially resistant to cleavage.

Exonuclease III activity depends partially on helical structure and displays sequence dependency (C>A=T>G). Temperature, salt concentration, and the ratio of enzyme to DNA greatly affect enzyme activity, requiring reaction conditions to be tailored to specific applications.

Exonuclease III has also been reported to have RNase H, 3´-phosphatase, and AP-endonuclease activities.

It is applicable to:

- Unidirectional nested deletions
- Site-directed mutagenesis
- Preparation of strand-specific probes
- Preparation of single-stranded substrates for dideoxy sequencing

Product Source:

Purified from *E. coli* strain carries *E. coli* exonuclease III gene.

Unit Definition:

One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble total nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X ABuffer A with 0.15 mM sonicated duplex [^{3}H]-DNA.

Reaction Conditions:

1X ABuffer A, Incubate at 37°C

1X ABuffer A:

10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7 @ 25°C

Storage Temperature: -20°C

Storage Conditions:

5 mM KPO_4, 200 mM KCl, 5 mM β-ME, 0.05 mM EDTA, 200 µg/ml BSA, 50% Glycerol, pH 6.5 @ 25°C

Heat Inactivation: 70°C for 20 min

Specific Activity: 150,000 units/mg

Notes:

Phosphorothioate linkages are not cleaved by Exonuclease III. Unidirectional deletions can also be created by protecting one terminus by incorporation of α-phosphorothioate-containing nucleotide.

Activity in ABclonal Buffer:

ABufferA	ABufferB	ABufferC	ABufferS	CutS
100%	75%	25%	75%	100%

QC Process:

- Purity is above 95% detected by SDS-PAGE.
- No endonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.