Exonuclease III (*E.coli*)

Cat. No. : RK20533

Product components

Components	Component number	Concentration	Size-1 5,000 U	Size-2 25,000 U
Exonuclease III (*E.coli*)	RM20521	100,000 U/mL	50 μL	250 μL
10X ABuffer A	RM20125	10X	1.25 mL	1.25 mL

Product Description

Exonuclease III (*E.coli*) catalyzes the stepwise removal of mononucleotides from 3´-hydroxyl termini of duplex DNA. Every time ExoIII enzyme binds to dsDNA, a few nucleotides are removed, leading to different degrees of progressive end deletions in the molecular population of DNA.

The preferred substrates are dsDNA with blunt or recessed 3´-,termini although the enzyme also acts at nicks in dsDNA to produce single-strand gaps. dsDNA with 3´-overhang are resistant to cleavage; when overhang's length is ≥ 4 bases, dsDNA are able to fully prevent ExoIII cleavage process.

Exonuclease III activity partially depends on helical structure and has sequence dependency (C>A=T>G). Temperature, salt concentration and enzyme: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

Storage

-20℃

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℃

1X ABuffer A

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

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Storage Conditions

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

Heat Inactivation

70°C for 20 min

Activity in ABclonal Buffer

ABuffer A	ABuffer B	ABuffer C	ABuffer S	CutS
100%	75%	25%	75%	100%

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.

Operation Description

Follow the steps below for the digestion reaction.

1. Set-up the reaction as follows:

Components	50 μL Reaction
DNA	~5 μg
Exonuclease III (*E.coli*)	0.5 μL (50 U)
10X ABuffer A	5 μL
Nuclease-Free-Water	to 50 μL

2. Incubate at 37°C for 30 minutes.
3. Terminate reaction by adding EDTA to 11 mM.
4. Heat inactivation 70°C for 30 minutes.
5. To purify digested samples, we recommend using one of the following steps:

① Column clean up or Agarose gel recovery.

② Performing a phenol/chloroform extraction followed by ethanol precipitation.

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