$RecJ_f$ Exonuclease

REF: EG26205-S/M

Storage Condition

Store at -20℃ for 2 years.

Components

Component	EG26205S	EG26205M
$RecJ_f$ Exonuclease (30 U/μl)	50 μl	250 μl
10× Cut Buffer G	1.5 ml	1.5 ml

Description

$RecJ_f$ Exonuclease is an Mg^{2+}-dependent, single-stranded DNA-specific exonuclease that degrades single-stranded DNA in the 5'→3' direction. $RecJ_f$ Exonuclease is a recombinant fusion protein of RecJ and maltose-binding protein (MBP), expressed and purified from E. coli, and exhibits the same enzymatic properties as wild-type RecJ. $RecJ_f$ Exonuclease can degrade 5' overhangs longer than 6 nt in double-stranded DNA, though the products are not all blunt-ended. It does not require 5'-terminal phosphorylation for activity, and it cannot digest RNA.

Definition of Activity Unit

One unit (U) is defined as the amount of enzyme that catalyzes the production of 0.05 nmol of TCA-soluble deoxyribonucleotides from single-stranded DNA in 30 min at 37℃ in a 50 μl reaction system.

Applications

1. Degradation of single-stranded DNA from the 5' end, and partial removal of 5' single-stranded overhangs from double-stranded DNA (does not produce fully blunt-ended dsDNA);

2. Use in homologous recombination, base excision repair, and methyl-directed mismatch repair.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 30 U of $RecJ_f$ Exonuclease incubated for 4 hours at 37℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 30 U of $RecJ_f$ Exonuclease incubated for 16 hours at 37℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 30 U of $RecJ_f$ Exonuclease incubated for 1 hour at 37℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Protocol

① Prepare the following reaction mixture on ice:

Reagent	Amount
ssDNA	X μl (≤1 μg)
10× Cut Buffer G	5 μl
$RecJ_f$ Exonuclease (30 U/μl)	1 μl
ddH_2O	Up to 50 μl

② Mix gently and spin down.

③ Incubate at 37℃ for 20~60 min; the reaction time may be extended as appropriate.

④ Terminate the reaction by incubation at 65℃ for 5 min.

Notice

1. Since $RecJ_f$ Exonuclease specifically catalyzes the degradation of linear single-stranded DNA in the 5'→3' direction, and cannot degrade single-stranded DNA in the 3'→5' direction nor act on double-stranded DNA, please select the appropriate substrate type.

2. Phosphorothioate (PT) bonds hinder $RecJ_f$ Exonuclease cleavage, but only one of the two chiral configurations of the PT bond blocks cleavage. To completely prevent degradation at all PT positions, it is recommended to include five consecutive PT bonds in chemically synthesized DNA.

3. It is not recommended to use $RecJ_f$ Exonuclease for preparing blunt-ended DNA, because when the 5' overhang of double-stranded DNA is only 6 or fewer bases, $RecJ_f$ Exonuclease does not effectively exhibit its 5'→3' exonuclease activity.

4.This product is for research use only and not intended for any other purpose.

5. For your safety and health, please wear a lab coat, disposable gloves, and a mask while conducting the experiment.

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