Exonuclease I

REF: EG24213-S/M

Storage Condition

-20℃

Components

Component	EG24213S	EG24213M
Exonuclease I (20 U/μl)	75 μl	375 μl
10× Exo I Buffer	1 ml	5×1 ml

Description

Exonuclease I (*E. coli*) is a single-strand specific exonuclease that degrades single-stranded DNA in the 3'-5' direction, releasing 5'-deoxyribonucleotides. Exo I exhibits strong specificity for single-stranded DNA and does not degrade double-stranded DNA or RNA. Additionally, it cannot degrade single-stranded DNA with 3'-OH terminiblocked by phosphoryl or acetyl groups. This product is obtained by recombinant expression of the Exo I gene (*E. coli*) in Escherichia coli, followed by multiple purification steps.

Definition of Activity Unit

One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in 30 minutes at 37 ℃ in 1× Exonuclease I Reaction Buffer with single-stranded DNA.

Applications

1. Removing primers and oligonucleotides from PCR products: Exo I can remove the unused primers in the PCR reaction as well as the excess single-stranded DNA generated during the amplification process.

2. Remove ssDNA from the nucleic acid mixture: Removal of linear single-stranded DNA, leaving behind double-stranded DNA in the sample.

3. Detection of single-stranded DNA (ssDNA): Exo I can be used to detect ssDNA with a free 3'-hydroxyl group.

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 100 U of Exonuclease I incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 100 U of Exonuclease I 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 100 U of Exonuclease I incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

Using the third method of the determination of exogenous DNA residue in General Chapter 3407 of ChP(2020) Volume IV, the residual amount of Escherichia coli host cell DNA in this product is less than 1 copy per 20 units.

Protocol

1. Recommended reaction system

(1) Primer or other ssDNA residues removal from PCR product:

Reagent	Amount
PCR mixture	5 μl
Exonuclease I	0.5 μl (10 U)

Note: In combination with Shrimp Alkaline Phosphatase (SAP), it is used to remove the remaining deoxynucleotide triphosphates (dNTPs). Consequently, there is no need to purify the PCR products, and the sequencing reaction can be carried out directly.

(2) Removal of single-stranded DNA

Reagent	Amount
DNA mixture[a]	2 μg
Exonuclease I	1 μl
10× Exo I Buffer	2 μl
ddH_2O	Up to 20 μl

a: The amount of single-stranded DNA in the DNA mixture should not exceed 1 μg.

2. Recommended reaction conditions

Incubation at 37℃ for 15~30 min。

3. Heat Inactivation

Incubation at 80℃ for 20 min。

Notice

1. Exo I is compatible with most PCR systems and can be directly added to the PCR products. The purified PCR products can be directly used for sequencing, but it is not recommended for direct use in cloning.

2. Exo I is used to remove ssDNA alone, it is recommended to use it in conjunction with 10× Exo I Buffer.

3. Exo I cannot cleave double-stranded DNA. Therefore, single-stranded DNA with secondary structures needs to be denatured before it can be completely digested.

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

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