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roboklon

Exonuclease I

Escherichia coli

Exonuclease I
Exo I, Escherichia coli

Cat. No.	Size
E1150-01	4.000 units
E1150-02	20.000 units

Unit Definition:
One unit is defined as the amount of enzyme that catalyses the release of 10 nmol acid-soluble nucleotides in a total reaction volume of 50 µl within 30 minutes at 37˚C.

Storage Conditions:
Store at -20˚C

Enzyme Inactivation: 15 min @ 80˚C

Quality Control:
All preparations are assayed for contaminating RNase, endonuclease, and double-stranded exonuclease activities.

Catalyzes removal of nucleotides from single-stranded DNA in 3' → 5' direction.

Applications:

- → Removal of residual ssDNA, including oligonucleotides ("oligos"), from reaction mixtures.
- → Does not degrade double-stranded DNA or RNA.
- → Requires magnesium and presence of free, accessible 3'-hydroxyl-termini.
- → Active in a wide variety of buffer conditions, allowing for direct addition of enzyme to most reaction mixtures.

Exonuclease I - Standard PCR Clean-up Protocol:

Mix the following reaction components:

25-50 µl of PCR just after amplification
0.5 µl 10 U Exonuclease I
1 µl 5 U Polar BAP (Cat. No. E1027)

Incubate for 15 min at 37˚C
Heat inactivation: 15 min at 80˚C

Up to 5 µl may be used directly to sequencing without any other purification.
For sequencing purposes it is recommended to use PCR devoid of any non-specific products
No specific buffers are required. Stable reaction performance is maintained in a broad variety of assay buffer conditions.

10 x Reaction Buffer:

670 mM Glycine-KOH (pH 9.5 @ 25˚C), 100 mM 2-mercaptoethanol, 67 mM $MgCl_2$.

Assay Conditions (Quality Control):

67 mM Glycine-KOH (pH 9.5 @ 25˚C), 10 mM 2-mercaptoethanol, 6.7 mM $MgCl_2$, 0.17 mg/ml single-stranded [^{3}H]-DNA. Incubation is at 37˚C for 10 min in a reaction volume of 50 µl.

References:

1. *Lehman and Nussbaum (1964) J. Biol. Chem., 239, 2628.*
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3. *Kushner, S.R. et al. (1972) Proc. Natl. Acad. Sci. USA 69, 1366.*
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5. *Rosamond et al. (1979) J. Biol. Chem., 254, 8646*
6. *Werle et al. (1994) Nuc. Acids Research 22(20): 4354-4355*