

Manual

Exonuclease V (RecBCD)

Recombinant enzyme that cleaves linear single-stranded and double-stranded DNA in both 3' to 5' and 5' to 3' directions. Concentration $10 \text{ U/}\mu\text{L}$.

| catalog# | size |
|-----------|--------|
| 1036-1000 | 1000 U |
| 1036-5000 | 5000 U |

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.

The company does not guarantee correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Advantages

- DNA specific exonuclease that has ssDNA and dsDNA exonuclease activity and ATP-dependent ssDNA endonuclease activity.
- Cleaves ssDNA and dsDNA in both 3' to 5' and 5' to 3' directions.
- Degrades linear ssDNA and dsDNA leaving intact supercoiled and circular dsDNA.

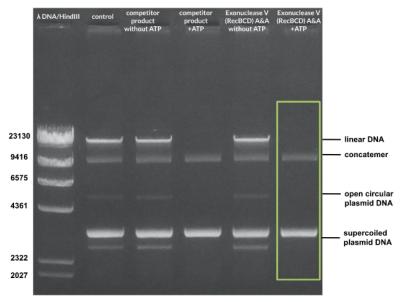


Fig. 1 The picture shows the degradation of linear DNA by Exonuclease V (RecBCD) A&A compared to a competitor enzyme.

Description

Exonuclease V is a complex of recombinant RecB, RecC and RecD proteins from $E.\ coli.$ Enzyme has a helicase and nuclease activity. In the presence of ATP catalyzes exonucleolytic cleavage in both 3' to 5' and 5' to 3' directions to yield 5' phosphooligonucleotides.

Application

- Removal of residual fragmented genomic DNA after purification of low copy plasmid.
- Removal of genomic DNA during purification of mitochondrial and chloroplast DNA.
- Removal of linear DNA contamination from plasmid samples.

Unit definition

One unit is defined as the amount of enzyme required to produce 1 nmol of acid soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37 °C in a total reaction volume of $30 \,\mu$ l.

Contents

| | quantity | catalog# | quantity | catalog# | storage |
|--|----------|-------------|------------|-------------|----------|
| Exonuclease V storage buffer: 100 mM MOPS pH 7.4, 2 mM MgCl ₂ , 1 mM DTT, 0.01 mM EDTA, 50% glycerol | 1000 U | K-EXV-1000 | 2 x 2500 U | K-EXV-2500 | -20 °C |
| ExoV reaction buffer 10x ExoV reaction buffer: 500 mM CH ₃ COOK, 200 mM Tris-acetate pH 7.9, 100 mM (CH ₃ COO) ₂ Mg, 10 mM DTT | 200 µl | K-BEXV-200B | 5 x 200 μl | K-BEXV-200B | -20 °C |
| 10 mM ATP | 200 µl | K-ATP-200B | 5 x 200 µl | K-ATP-200B | -20 °C |
| ultrapure water | 8 ml | K-WUP-8 | 40 ml | K-WUP-40 | -20-25 ℃ |

Important note

DNA treated with Exonuclease V must be free of **EDTA** and **CaCl₂**. We recommend suspending the material in water or Tris buffer.

Protocol

1. Thaw and mix all components and add:

| | reaction volume |
|----------------------|-----------------|
| component | 30 μΙ |
| DNA sample | up to 1 µl |
| Exonuclease V | 1 μΙ |
| ExoV reaction buffer | 3 μΙ |
| ATP (10 mM) | 3 μΙ |
| ultrapure water | up to 30 µl |

- Incubate for 30 min at 37 °C.
- 4. Enzyme inactivation: add EDTA to 11mM or incubate for 30 min at 70 °C.
- 5. Clean-up treated samples using the Clean-Up Concentrator cat # 021-50C and/or perform ethanol precipitation.



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