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RecBCD Nuclease

Cat. No. BCD0401K

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1. Introduction

RecBCD Nuclease selectively hydrolyzes linear double-stranded (ds) DNA to deoxynucleotides at slightly alkaline pH, and with a lower efficiency, linear and closed-circular single-stranded DNAs.[1] The reaction is ATP-dependent, and does not affect closed circular supercoiled or nicked circular dsDNAs. The enzyme can be conveniently and completely heat-inactivated by a 30 minute incubation at 70°C.

RecBCD Nuclease is available in a 1,000 unit size at a concentration of 10 U/µl. The enzyme is supplied with a 10X Reaction Buffer and a 25 mM ATP Solution.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: RecBCD Nuclease is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT) and 0.1% Triton® X-100.

Unit Definition: One unit degrades one nmol of deoxynucleotides in linear dsDNA in 30 minutes at 37°C in 1X RecBCD Nuclease Reaction Buffer and 1 mM ATP.

RecBCD Nuclease 10X Reaction Buffer: is 330 mM Tris-acetate (pH 7.5 at 25°C), 660 mM potassium acetate, 100 mM magnesium acetate and 5.0 mM DTT.

ATP is required for RecBCD Nuclease activity and should be added to a final concentration of 1 mM.

Contaminating Activity Assays: RecBCD Nuclease is free of detectable RNase and double-strand-specific endonuclease activities.

3. Related Products

The following products are also available:

– 10 mM ATP Solution
– Ready-Lyse™ Lysozyme Solution

4. Protocol for RecBCD Nuclease

Enzymatic activity requires a slightly alkaline pH, 10 mM Mg^{++} and 1 mM ATP. Digestions with RecBCD Nuclease can be performed directly in many common enzyme buffers that have been supplemented with ATP. We recommend that RecBCD Nuclease digestion be performed after the completion of a standard nucleic acid isolation procedure.

RecBCD Nuclease Protocol

1. Isolate DNA from overnight bacterial cultures using standard protocols.
2. Resuspend the DNA in:

42 µl	sterile water
2 µl	25 mM ATP
5 µl	10X Reaction Buffer
1 µl	RecBCD Nuclease (10 U)
50 µl	Total Reaction Volume

3. Incubate at 37°C for 30 minutes
4. Inactivate RecBCD Nuclease by incubation at 70°C for 30 minutes.
   Note: *Treated DNA can be further purified by ethanol precipitation, spin-columns or organic extraction.*

5. Reference

1. Boehmer, P.E. and Emmerson, P.T., (1991) *Gene* **102**, 1.

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