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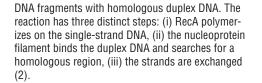




200 μg 2 mg/ml Lot: 0021309 RECOMBINANT Store at -20°C Exp: 9/15

**Description:** *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis. RecA promotes the autodigestion of the lexA repressor, umuD protein and lambda repressor. Cleavage of LexA derepresses more than 20 genes (1). *In vitro* studies indicate that in the presence of ATP, RecA promotes the strand exchange of single-strand

**Purified with Affinity Tag** 



**Source:** RecA<sub>f</sub> is expressed as a N-terminal 6X His tagged recombinant protein from a plasmid in *E. coli* strain ER3010 which encodes a full-length 353 amino acids wild type *E. coli* RecA protein.

Supplied in: 0.1 mM EDTA, 10 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM DTT and 50% glycerol.

#### **Applications:**

- Visualization of DNA structures with electron microscopy (3)
- D-loop mutagenesis (4)
- Screening libraries using RecA-coated probes (5,6)
- Cleavage of DNA at any single predetermined site (7,8,9)
- RecA mediated affinity capture for full length cDNA cloning (10,11)

#### Reagents Supplied with Enzyme:

10X RecA Reaction Buffer.

**Note:** ATP $\gamma$ S which is required for triple helix formation is not supplied.

#### Reaction Conditions:

1X RecA Reaction Buffer. Incubate at 37°C.

#### 1X RecA Reaction Buffer:

70 mM Tris-HCl 10 mM MgCl<sub>2</sub> 5 mM dithiothreitol pH 7.5 @ 25°C

**Unit Definition:** Protein concentration is determined by the Pace method (12) using extinction coefficient of 21.555 for recA

Molecular Weight: 38,796 daltons.

Heat Inactivation: 65°C for 20 minutes.

**Quality Assurance:** Each lot is tested for its ability to form a stable triple helix and is visually determined to be > 99% pure on an SDS-polyacrylamide gel.

#### **Quality Control Assays**

**Exonuclease Activity:** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and 10 µg of RecA<sub>f</sub> incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

**Endonuclease Activity:** A 50  $\mu$ I reaction in RecA Reaction Buffer containing 1  $\mu$ g of supercoiled  $\phi$ X174 RF I DNA and 10  $\mu$ g of RecA<sub>f</sub> incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Non-specific Nuclease Assay: A 50  $\mu$ l reaction in RecA Reaction Buffer containing 1  $\mu$ g  $\lambda$  DNA and 10  $\mu$ g of RecA<sub>f</sub> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**RNase Assay:** A 50  $\mu$ I reaction in RecA Reaction Buffer containing 40 ng of labeled RNA and 10  $\mu$ g of RecA<sub>f</sub> is incubated at 37°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

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CERTIFICATE OF ANALYSIS

## RecA<sub>f</sub>



1-800-632-7799 in f o @ n e b. c o m w w w. n e b. c o m

# M03558 002130915091

### M0355S



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**Purified with Affinity Tag** 

DNA fragments with homologous duplex DNA. The reaction has three distinct steps: (i) RecA polymerizes on the single-strand DNA, (ii) the nucleoprotein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged (2).

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(see other side)

CERTIFICATE OF ANALYSIS

**RecA Functional Assay:** The plasmid pUC19 contains 5 HpyCH4IV sites. A 60 mer was designed with complementarity to the region centered around the HpyCH4IV site at position 374. A reaction containing 1 µg pUC19, 0.18 µg 60 mer, 0.3 mM ATP γ-S, 4 μg RecA<sub>f</sub>, in 40 μl 1X RecA Reaction Buffer was incubated at 37°C for 10 minutes to form a stable triple helix. The unprotected sites were methylated using 8 units of SssI supplemented with 160 µM SAM for 10 minutes at 37°C. The reaction was stopped and the triple helix was disrupted by incubation at 65°C for 15 minutes. The reaction was cooled and 10 units of HpyCH4IV were added followed by digestion at 37°C for 20 minutes. > 95% of the product is single cut pUC19.

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#### References:

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- 10. Zhumabayeva, B. et al. (1990) *Biotechniques* 27, 834–845.
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- 12. Pace, C.N. et al. (1995) *Protein Sci.* 4, 2411–2423.

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