

RecA_f



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M0355S 002150117011

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200 µg **2 mg/ml** **Lot: 0021501**
RECOMBINANT **Store at -20°C** **Exp: 1/17**

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

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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).

Source: RecA_f 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)

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Reagents Supplied with Enzyme:

10X RecA Reaction Buffer.

Note: ATP_γS which is required for triple helix formation is not supplied.

Reaction Conditions:

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

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70 mM Tris-HCl
10 mM MgCl₂
5 mM dithiothreitol
pH 7.5 @ 25°C

Molecular Weight: 38,907 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.

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Quality Control Assays

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

Endonuclease Activity: A 50 µl reaction in RecA Reaction Buffer containing 1 µg of supercoiled φX174 RF I DNA and 10 µg of RecA_f 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 µl reaction in RecA Reaction Buffer containing 1 µg λ DNA and 10 µg of RecA_f 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 µl reaction in RecA Reaction Buffer containing 40 ng of labeled RNA and 10 µg of RecA_f is incubated at 37°C. After incubation for 16 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

Molecular Weight Determination (Mass Spectrometry):

The molecular weight of RecA_i is between 38,897 and 38,917 as determined by mass spectrometry analysis.

Protein Concentration Determination:

The concentration of RecA_i is between 1.9 and 2.1 mg/ml as determined by UV absorption at 280 nm by the Pace method using the extinction coefficient of 21,555 and molecular weight of 38,907 daltons (12).

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_i, 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

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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.

References:

1. West, S.C. (1992) *Ann. Rev. Biochem.* 61, 603–640.
2. Radding, C.M. (1991) *J. Biol. Chem.* 266, 5355–5358.
3. Wasserman, S.A. and Cozzarelli, N.R. (1985) *Proc. Natl. Acad. Sci. USA* 82, 1079–1083.
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