

RecA_f



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M0355S 001130415041

M0355S



200 µg **2 mg/ml** **Lot: 0011304**

RECOMBINANT **Store at -20°C** **Exp: 4/15**

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

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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 γ 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 MgCl₂
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.

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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

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_r, 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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