RecA$_f$

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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www.neb.com

M0355S 002160118011

M0355S

RR Yes

200 µg	**2 mg/ml**	**Lot: 0021601**
RECOMBINANT	**Store at –20°C**	**Exp: 1/18**

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 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).

Purified with Affinity Tag

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)

Reagents Supplied with Enzyme:
10X RecA Reaction Buffer.

Note: ATPγS 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 $MgCl_2$
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.

Quality Control Assays

Exonuclease Activity: A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *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 $RecA_f$ is between 38,897 and 38,917 as determined by mass spectrometry analysis.

Protein Concentration Determination: The concentration of $RecA_f$ 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 $RecA_f$, 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.

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.
4. Shortle, D. et al. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5375–5379.
5. Honigberg, S.M. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9586–9590.
6. Rigas, B. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9591–9595.
7. Ferrin, L.J. and Camerini-Otero, R.D. (1991) *Science* 254, 1494–1497.
8. Koob, M. et al. (1992) *Nucleic Acids Res.* 20, 5831–5836.
9. Koob, M. (1992). In R. Wu (Ed.), *Methods in Enzymology* Vol. 216, (pp. 321–329). San Diego: Academic Press.
10. Zhumabayeva, B. et al. (1990) *Biotechniques* 27, 834–845.
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