RecA

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

M0249S 023121214121

M0249S

RR Yes

200 µg **2 mg/ml** **Lot: 0231212**
RECOMBINANT **Store at –20°C** **Exp: 12/14**

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.

Now Supplied With Buffer

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: An *E. coli* strain ER2502 that carries an overexpressed RecA gene from *E. coli*.

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)

Supplied in: 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

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 7.6 @ 25°C

Unit Definition: Sold by mass of pure protein as determined by OD_{280} (A_{280} = 0.516 at 1 mg/ml, 1 cm).

Molecular Weight: 37,842 daltons.

Quality Assurance: RecA is purified free of contaminating endonucleases and exonucleases. Each lot is tested for its ability to form a stable triple helix and is visually determined to be > 95% pure on an SDS-polyacrylamide gel.

Quality Control Assays

Exonuclease Activity: Incubation of 4 µg RecA for 4 hours at 30°C in 50 µl 1X reaction buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol and 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) released 0.2% acid soluble counts.

Endonuclease Activity: Incubation of 10 µg RecA for 4 hours at 30°C in 50 µl reaction buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol and 1 µg ϕX174 RF I DNA gave < 5% conversion to RF II.

Nuclease Activity: Incubation of 6 µg RecA for 16 hours at 30°C in 50 µl of reaction buffer containing 20 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol and 1 µg λ DNA yielded a clear and sharp band on an agarose gel.

Ribonuclease Activity: Incubation of 6 µg RecA with 2 µg MS2 phage RNA for 1 hour at 30°C in 50 µl 1X T4 Polynucleotide Kinase Buffer followed by agarose gel electrophoresis gave no change in banding.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)
CERTIFICATE OF ANALYSIS

RecA Functional Assay: The plasmid pUC19 contains 5 HpyCH4 IV sites. A 60 mer was designed with complementarity to the region centered around the HpyCH4 IV site at position 374. A reaction containing 1 µg pUC19, 0.18 µg 60 mer, 0.3 mM ATP γ-S, 4 µg RecA, 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 Sss I 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 HpyCH4 IV were added followed by digestion at 37°C for 20 minutes. > 90% of the product is single cut pUC19.

Heat Inactivation: 65°C for 20 minutes.

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.
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Page 2 (M0249)