

RecA



1-800-632-7799
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M0249S 022120614061

M0249S



200 µg **2 mg/ml** **Lot: 0221206**
RECOMBINANT **Store at -20°C** **Exp: 6/14**

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

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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.

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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.

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70 mM Tris-HCl
10 mM MgCl₂
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pH 7.6 @ 25°C

Unit Definition: Sold by mass of pure protein as determined by OD₂₈₀ (A₂₈₀ = 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.

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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 MgCl₂, 1 mM dithiothreitol and 1 µg sonicated [³H] DNA (10⁵ 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 MgCl₂, 1 mM dithiothreitol and 1 µg ϕ X174 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 MgCl₂, 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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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.

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