

RecA



M0249S



200 µg **2 mg/ml** **Lot: 0281612**
RECOMBINANT **Store at -20°C** **Exp: 12/18**

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).

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: 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 7.6 @ 25°C

Molecular Weight: 37,973 daltons.

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 (Radioactivity Release): 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 incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Endonuclease Activity (Nicking): A 50 µl reaction in RecA Reaction Buffer containing 1 µg of supercoiled ϕ X174 RF I DNA and 10 µg of RecA incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 hour): A 50 µl reaction in RecA Reaction Buffer containing 1 µg of λ DNA and 10 µg of RecA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

RNase Activity (Extended Digestion): A 50 µl reaction in RecA Reaction Buffer containing 40 ng of labeled RNA and 10 µg of RecA is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel.

Molecular Weight Determination (Mass Spectrometry): The molecular weight of RecA is between 37,963 and 37,983 as determined by mass spectrometry analysis.

(see other side)

CERTIFICATE OF ANALYSIS

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Molecular Weight Determination (Mass Spectrometry): The molecular weight of RecA is between 37,963 and 37,983 as determined by mass spectrometry analysis.

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CERTIFICATE OF ANALYSIS

Protein Concentration Determination: The concentration of RecA 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 37,973 daltons (12).

RecA Functional Assay (Triple Helix Formation):

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.

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References:

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2. Radding, C.M. (1991) *J. Biol. Chem.* 266, 5355–5358.
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