RecA

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: 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 MgCl2
5 mM dithiothreitol
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.

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 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.

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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.

Heat Inactivation: 65°C for 20 minutes.

References: