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)
- RecAmediated 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.

Nuclease Activity: Incubation of 6 µg RecA for 16 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.
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: