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: RecA is expressed as a N-terminal 6X His tagged recombinant protein from a plasmid in E. coli strain ER3010 which encodes a full-length 353 amino acids wild type E. coli RecA protein.

Supplied in: 0.1 mM EDTA, 10 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM DTT and 50% glycerol.

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)

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 7.5 @ 25°C

Unit Definition: Protein concentration is determined by the Pace method (12) using extinction coefficient of 21,555 for recA

Molecular Weight: 38,796 daltons.

Heat Inactivation: 65°C for 20 minutes.

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: 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: A 50 μl reaction in RecA Reaction Buffer containing 1 μg of supercoiled 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 Nuclease Assay: 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 16 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

RNase Assay: 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 16 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

(see other side)
RecA Functional Assay: The plasmid pUC19 contains 5 HpyCH4IV sites. A 60 mer was designed with complementarity to the region centered around the HpyCH4IV 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 SssI 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 HpyCH4IV were added followed by digestion at 37°C for 20 minutes. > 95% of the product is single cut pUC19.

References: