

rec A Protein

Cat. Nos. RC44200 and RC441MG

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1. Introduction

rec A Protein is a multifunctional DNA-binding protein isolated from *E. coli*, involved in both homologous recombination and post-replication DNA repair mechanisms. *In vitro*, *rec A* Protein catalyzes homologous base-pairing and strand displacement through a multiple step ATP-dependent pathway. Initially, the protein binds preferentially to single-stranded (ss) DNA forming a nucleoprotein filament.¹ The filament complex then binds naked duplex DNA and searches for homologous sequences.¹ When a region of homology is found, strand displacement and exchange begins.¹ Substitution with the non-hydrolyzable ATP analog, ie. ATP[γ S], arrests the displacement reaction at the triple-stranded intermediate step.²

2. Applications

Site-directed mutagenesis through displacement loop structures.³ A single-stranded homologous DNA fragment coated with *rec A* Protein is mixed with target duplex DNA. At the site of *rec A* Protein-catalyzed homologous alignment, one strand of the target DNA is displaced, creating a local single-stranded loop in the region of the target DNA to be mutated. Treatment with S1 nuclease nicks the displaced strand. Nick sites are then used as the origins for the insertion of deletion mutations.

Targeted site-specific cleavage of DNA.⁴⁻⁶ An oligonucleotide coated with *rec A* Protein is designed to be homologous to the immediate region surrounding a targeted restriction site. The complex formation catalyzed by *rec A* Protein protects the restriction site from DNA methylation. After removal of the *rec A* Protein-coated oligonucleotide, only the protected site will be unmethylated and thus susceptible to subsequent restriction enzyme digestion. This technique is known as *rec A*-Assisted Restriction Endonuclease (RARE) Cleavage or *rec A*-mediated Achilles' Cleavage (Rec A-AC). It can be used on genomic sequences that are difficult to clone into YACs or cosmids.

Enrichment of target sequences from libraries or other DNA pools.^{7,8} *rec A* Protein, coated to a ssDNA, forms a probe capable of selectively binding to homologous sequences in DNA. The DNA can then be used to concentrate target sequences in heterogeneous mixtures of DNA such as cDNA libraries. After incubation and binding of the *rec A* Protein-coated probe to the homologous sequences, bound DNA is concentrated by selective centrifugation of reaction aggregates, or through affinity selection of tagging groups present on the probe DNA.

Visualization of DNA for electron microscopy.⁹ Naked DNA is coated with *rec A* Protein to increase the width and length of the DNA molecules allowing easier detection under scanning electron microscopy analysis.

3. Product Specifications

Storage: Store only at -20°C in a freezer without a defrost cycle.

Storage Buffer: *rec A* Protein is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1% Triton[®] X-100.

Purity: *rec A* Protein is $>95\%$ pure as determined by SDS-PAGE analysis.

Contaminating Activity Assays: *rec A* Protein is certified free of contaminating RNase, DNase, and protease activities.

4. References

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