

## **rec A Protein**

Cat. Nos. **RC44200** and **RC441MG**

**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.<sup>1</sup> The filament complex then binds naked duplex DNA and searches for homologous sequences.<sup>1</sup> When a region of homology is found, strand displacement and exchange begins.<sup>1</sup> Substitution with the non-hydrolyzable ATP analog, ie. ATP[ $\gamma$ S], arrests the displacement reaction at the triple-stranded intermediate step.<sup>2</sup>

### **Applications**

**Site-directed mutagenesis through displacement loop structures.**<sup>3</sup> 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.**<sup>4,6</sup> 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.**<sup>7,8</sup> **rec A Protein**, coated to a ssDNA, forms a probe capable of selec-

tively 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.**<sup>9</sup> 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.

### **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<sup>®</sup> 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.

### **References:**

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