Incubation of 20 µg RecA

36 kDa.

RecA is purified free of contaminants endonucleases and exonucleases.

Quality Assurance: RecA is purified free of contaminating endonucleases and exonucleases. Each lot is tested for single-stranded DNA-dependent ATPase activity and is visually determined to be > 95% pure on an SDS-polyacrylamide gel.

Applications:
- Visualization of DNA structures with electron microscopy (1)
- Site-directed mutagenesis through D-loop (2,3)
- Screening of DNA libraries using RecA-probe filaments (4,5)
- Targeted cleavage of DNA (6)

Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100 and 50% glycerol.

Unit Definition: Sold by mass of pure protein as determined by OD_{280}.

Molecular Weight: 36 kDa.

Quality Control Assays

Exonuclease Activity: Incubation of 20 µg RecA for 4 hours at 65°C in 50 µl reaction buffer containing 50 mM potassium acetate (pH 7.9), 20 mM tris-acetate, 10 mM magnesium acetate and 1 mM dithiothreitol (pH 7.9 at 25°C), with a mixture of single and double-stranded [3H] E. coli DNA (200,000 cpm/µg) released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of 2 µg RecA for 4 hours at 65°C in 50 µl reaction buffer containing 50 mM potassium acetate (pH 7.9), 20 mM tris-acetate, 10 mM magnesium acetate and 1 mM dithiothreitol (pH 7.9 at 25°C), with 1 µg φX174 RF I DNA gave < 20% conversion to RF II.

Nuclease Activity: Incubation of 20 µg RecA for 16 hours at 65°C in 50 µl of reaction buffer containing 50 mM potassium acetate (pH 7.9), 20 mM tris-acetate, 10 mM magnesium acetate and 1 mM dithiothreitol (pH 7.9 at 25°C), with 1 µg λ DNA yielded a clear and sharp band on an agarose gel.

Notes On Use: Tth RecA is active in any polymerase buffer. Add 400 ng of the Tth RecA per 50 µl reaction.

References:

Source: An E. coli strain that carries the cloned RecA gene from Thermus thermophilus. Developed and produced by Biohelix Corporation, a New England Biolabs-affiliated company.