Tth RecA

1-800-632-7799
info@neb.com
www.neb.com

M2402S 008140116011

M2402S

RR

100 µg **1 mg/ml** **Lot: 0081401**

RECOMBINANT **Store at –20°C Exp: 1/16**

Description: Tth RecA is a RecA homolog isolated from *Thermus thermophilus*. It has a ssDNA-dependent ATPase activity at an optimal temperature between 65–75°C. The extreme thermostability makes Tth RecA ideal for molecular biology applications that require an elevated temperature condition, such as nucleic acid amplification and sequencing.

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.

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 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.

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 [^{3}H] *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:

1. Wasserman, S.A. and Cozzarelli, N.R. (1985) *Proc. Natl. Acad. Sci. USA,* 82, 1079–1083.
2. Biet, E. et al. (2001) *Biochemistry,* 40, 1779–1786.
3. Shortle, D. et al. (1980) *Proc. Natl. Acad. Sci. USA,* 77, 5375–5379.
4. Rigas, B. et al. (1986) *Proc. Natl. Acad. Sci. USA,* 83, 9591–9595.
5. Honigberg, S.M. et al. (1986) *Proc. Natl. Acad. Sci. USA,* 83, 9586–9590.
6. Koob, M. et al. (1992) *Nucleic Acids Res.* 20, 5831–5836.
7. Shigemori, Y. et al. (2005) *Nucleic Acids Res.* e126.

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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.

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