

ET SSB



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M2401S 003121214121

M2401S



50 µg 500 µg/ml Lot: 0031212

RECOMBINANT Store at -20°C Exp: 12/14

Description: ET SSB (Extreme Thermostable Single-Stranded DNA Binding Protein) is a single-stranded DNA binding protein isolated from a hyperthermophilic microorganism, which remains fully active after incubation at 95°C for 60 minutes. Due to the extreme thermostability, ET SSB can be used in applications that require extremely high temperature conditions, such as nucleic acid amplification and sequencing.

Source: An *E. coli* strain that carries the cloned *ssb* gene from a hyperthermophilic organism.

Applications:

- Improve the processivity of DNA polymerase (1)
- Stabilization and marking of ssDNA structure (2)
- Increase the yield and specificity of PCR reactions (3–7)
- Increase the yield and processivity of RT during RT-PCR (8–9)
- Improve DNA sequencing through regions with strong secondary structure (6)
- Enhance the RecA activity for ssDNA binding and strand transfer (10,11)

Supplied in: 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.5 mM DTT, 1 mM EDTA and 50% glycerol.

Unit Definition: Sold by mass of pure protein as determined by OD₂₈₀ (A₂₈₀ = 0.774 at 1 mg/ml, 1cm).

Molecular Weight: 16 kDa.

Quality Assurance: ET SSB is purified free of contaminating endonucleases and exonucleases. Each lot is tested for ssDNA binding activity and is visually determined to be > 95% pure on an SDS-polyacrylamide gel.

Quality Control Assays

Exonuclease Activity: Incubation of 20 µg ET SSB for 4 hours at 65°C in 50 µl reaction buffer containing 50 mM potassium acetate, 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 7 µg ET SSB for 4 hours at 65°C in 50 µl reaction buffer containing 50 mM potassium acetate, 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 2.5 µg ET SSB for 16 hours at 65°C in 50 µl of reaction buffer containing 50 mM potassium acetate, 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: ET SSB is active in any polymerase buffer. Add 200 ng of ET SSB per 50 µl reaction.

References:

1. Myers, T.W. and Romano, L.J. (1988) *J. Biol. Chem.* 263, 17006–17015.
2. Reddy, M.S. (2000) *Biochemistry*, 39, 14250–14262.
3. West, S.C., Cassuto, E. and Howard-Flanders, P. (1982) *Mol. Gen. Genet.* 186, 333–338.
4. Goldmeyer, J., Kong, H. and Tang, W. (2007) *J. Mol. Diagnostics*, 9, 639–644.
5. Delius, H., Mantell, N.J. and Alberts, B. (1972) *J. Mol. Biol.* 67, 341–350.
6. Schwarz, K., Hansen-Hagge, T. and Bartram, C. (1990) *Nucl. Acids Res.* 18, 1079.
7. Chou, Q. (1992) *Nucl. Acids Res.* 20, 4371.
8. Oshima, R.G. (1992) *Biotechniques*, 13, 188.
9. Rapley, R. (1994) *Mol. Biotechnol.* 2, 295–298.
10. Olszewski, M. et al. (2005) *Mol. Cell Probes*, 19, 203–205.
11. Baugh, L.R. et al. (2001) *Nucl. Acids Res.* 29, e29.
12. Villalva, C. et al. (2001) *Biotechniques* 31, 81–83, 86.

CERTIFICATE OF ANALYSIS

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