# ET SSB

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M2401S 003160418041

# M2401S

**50 µg** **500 µg/ml** **Lot: 0031604**

**RECOMBINANT** **Store at –20°C** **Exp: 4/18**

**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 $OD_{280}$. ($A_{280}$= 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 [$^3$H] *E. coli* DNA (200,000 cpm/µg) released < 0.1% of the total radioactivity.

**Endonuclease Activity:** Incubation of 5 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.

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CERTIFICATE OF ANALYSIS

ET SSB

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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.






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Page 2 (M2401)