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)

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

Molecular Weight: 16 kDa.

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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.

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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 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 a mixture of ssDNA and dsDNA, no degradation was observed.

Notes On Use: ET SSB is active in any polymerase buffer. Add 200 ng of ET SSB per 50 µl reaction.

References: