



Product Information	
<i>E.coli</i> SSB	
Part Number:	Y903L
Lot Number:	

Rev.A

Product Description:

Single-Stranded DNA Binding Protein (SSB) preferentially binds single-stranded DNA, forming a tetramer of four identical 18.9 kDa subunits which protects 8-16 nucleotides, while not binding well to double-stranded DNA. In nature, SSB participates in DNA replication, recombination, and repair functions. *In vitro*, SSB has been found to stimulate certain DNA polymerase-mediated reactions by relaxing DNA secondary structure and enhancing enzyme processivity.

Source of Protein

A recombinant *E. coli* strain carrying the *E.coli* SSB gene.

Supplied in

50 mM Tris-HCl
200 mM NaCl
1.0 mM dithiothreitol
0.1 mM EDTA
50% glycerol
pH 7.5 @ 25°C

(additional information on reverse side)

Product Specification*	
Unit Size:	1.0 mg
Protein Concentration	5.0 mg/ml
Purity (SDS-PAGE)	>99%
SS Exonuclease	25 µg <0.1% release
DS Exonuclease	25 µg <0.1% release
Endonuclease	25 µg <10% converted
Functional Test	0.7 µg inhibits PCR
Storage	-20°C

* For a detailed summary of assay conditions and data, refer to the Quality Controls Analysis section below

Quality Control Analysis:

Catalytic Function Assay

Single-stranded DNA binding ability was confirmed in a PCR inhibition assay by adding decreasing amounts of *E.coli* SSB to a series of 50 µL PCR reactions containing 5 ng Lambda DNA, 200 µM dNTPs, 1X PCR buffer and Taq DNA Polymerase. Reactions were incubated in a thermal cycler and subjected to 25 PCR cycles. Samples were resolved using agarose gel electrophoresis and amount of SSB required to block 100% accumulation of PCR product was recorded. Acceptance criteria for assay: 0.70 µg *E.coli* SSB is required to inhibit PCR amplification of 5 ng Lambda DNA following 25 cycles of PCR.

Protein Concentration (OD₂₈₀) Measurement

A 3.0 µL sample of enzyme was analyzed at OD₂₈₀ using a Nanodrop ND-1000 spectrophotometer standardized using a 2.0 mg/ml BSA sample (Pierce Cat #23209) and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 27,880 and molecular weight of 18,970 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)

2.0 µL of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µL of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity

A 50 μ L reaction containing 15,000 cpm of a radiolabeled single-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity

A 50 μ L reaction containing 15,000 cpm of a radiolabeled double-stranded DNA substrate and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in less than 0.1% release of TCA-soluble counts.

Endonuclease Activity

A 50 μ L reaction containing 1 μ g of pBR322 DNA and 10 μ L of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.



Limitations of Use

This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. MSDS sheets relevant to this product are available upon request.