

Product Information	
<i>E. coli</i> SSB	
Part Number	Y9030L
Concentration	2.75 mg/mL
Unit Size	1.5 mg
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

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 (1,2,3).

Product Specifications					
Y9030					
Assay	SDS Purity	DNA Binding	SS Exonuclease	DS Exonuclease	DS Endonuclease
Units Tested	n/a	n/a	25 µg	25 µg	25 µg
Specification	>99%	0.7 µg inhibits PCR	<1.0% Released	<1.0% Released	No Conversion

Source of Protein: A recombinant *E. coli* strain carrying the *E. coli* *ssb* gene.

Molecular weight: 18.9 kDa

Quality Control Analysis:

Protein Concentration (OD₂₈₀) is determined by OD₂₈₀ absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

DNA Binding of single stranded DNA was confirmed in a PCR inhibition assay by adding decreasing amounts of *E. coli* SSB to a series of PCR reactions containing target 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 target DNA following 25 cycles of PCR.

Supplied in:

50 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol (pH 7.5 at 25°C)

Usage Instructions:

The *E. coli* SSB is a single-stranded DNA binding protein (SSB) which can be used for stabilization of single stranded regions of DNA and enhancement of DNA polymerase fidelity and processivity in PCR (1,5).

The protein can also be used to minimize deletion mutagenesis artifact during Taq DNA polymerase mediated PCR (4), improve yield of DNA amplification, and enable sequencing of problematic DNA templates, for example, regions with strong secondary structures (5).

As a starting point in SSB mediated PCR reactions, add *E. coli* SSB protein at a concentration range between 20–320 ng/μL per 50 μL reaction to identify the optimal concentration.

References:

1. Meyer, R.R. and Laine, P.S. (1990) *Microb. Rev.*, p342-380.
2. Krauss, G. et al. (1981) *Biochemistry.*, 20, 5346-52.
3. Weiner, J.H. et al. (1975) *J. Biol. Chem.*, 250, 1972-80.
4. Chou, Q. (1992) *Nuc. Acids. Res.*, 20(16), 4371.
5. Rapley, R. (1994) *Mol. Biotechnol.*, 2, 295-298.

Disclaimer:

Use of this enzyme in certain applications may be covered by patents and may require a license. Purchase of this product does not include a license to perform any patented application; therefore, it is the sole responsibility of the users of the product to determine whether they may be required to engage in a license agreement depending upon the particular application in which the product is used.

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. SDS sheets relevant to this product are available upon request.