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ProductInformation

Single-Strand Binding Protein
From *E. coli*

Product Code **S 3917**
Store at 0 to –20 °C
Synonym: SSB

Product Description

E. coli single-strand binding protein (SSB) is a thermostable single-strand specific DNA-binding protein thought to remove secondary structure from single-strand DNA.[1,2] In addition, it enhances the annealing of complimentary DNA strands. It has been shown to increase the processivity of DNA polymerases and facilitate DNA sequencing.[1,2] It also enhances both yield and processivity in PCR† for DNA templates that contain secondary structure and are prone to deletion mutagenesis.[3] Although SSB may rescue some problem PCR reactions, it may not be effective when processivity or secondary structure is not the problem. Use of the other components of the PCR Optimization Kit (Product Code OPT-2) may be helpful with problem PCR when SSB is not effective.

SSB is a homotetramer composed of four 18.9 kDa subunits.

Purity: >95% (SDS-PAGE).

Reagent

Storage Buffer for SSB: 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.1 mM EDTA, 0.1 mM DTT, and 50% glycerol. Concentration for each lot is given on the product label.

Procedure

As a starting point, for a standard 50 µl PCR containing 100-200 ng template, add 0.75-1.5 µg SSB. Further adjustment may be necessary. When the concentration of SSB is too low, it will have no effect and excess SSB may inhibit the PCR.

Product Profile

Endonuclease-Exonuclease (DNase)

1 µg of λ *Hin*d III fragments was incubated for 4 hours at 37 °C with SSB at a final concentration of 0.5 µg in a 50 µl reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% (w/v) gelatin. No degradation of the DNA fragments was detected by agarose gel electrophoresis.

Endonuclease (Nickase)

1 µg of pBR322 DNA was incubated for 16 hours at 37 °C with SSB at a final concentration of 0.5 µg in a 50 µl reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% (w/v) gelatin. No degradation of the DNA was detected by agarose gel electrophoresis.

RNase

2 µg of transfer RNA were incubated for 16 hours at 37 °C with SSB at a final concentration of 0.5 µg in a 50 µl reaction mixture containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% (w/v) gelatin. No degradation of the tRNA was detected by polyacrylamide gel electrophoresis.

Gel Shift Assay

M13mp18 (+ strand) was incubated at room temperature with varying concentrations of SSB (100-1500 ng) for 45 minutes. 10 µl of gel loading solution containing 0.05% (w/v) bromophenol blue and 40% (w/v) sucrose was added to each sample. The samples were run on a 0.85% agarose gel for 2 hours at 70 volts. The gel was stained with ethidium bromide and observed under UV light. The samples incubated with the SSB displayed a shift upward in the migration pattern when compared to the control DNA (containing no SSB), confirming binding of SSB to the DNA.

References

1. Kieleczawa, J., *et al*., Science **258**, 1787 (1992).
2. Sambrook, J., *et al*., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, p. 5.87 (1989).
3. Chou, Q., Nucleic Acids Res., **20**, 4371 (1992).

† The PCR(polymerase chain reaction) process is covered by patents owned by Hoffmann-LaRoche, Inc.

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