ProductInformation

SINGLE STRAND BINDING PROTEIN From *E. coli*

Product No. **S3917**

Lot No. 049H0582

Storage: 0 to -20°C

PRODUCT SUMMARY

E. coli single strand binding protein (SSB) is a homotetramer composed of four 18.9 kd subunits.

Storage Buffer: 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 0.1 mM EDTA, 0.1 mM DTT, 50% glycerol.

Concentration: 0.36 mg/ml

INTRODUCTION

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 PCR†(both yield and processivity) 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. Using the other components of the PCR Optimization Kit(Product No. KR654) may be helpful with problem PCR when SSB is not effective.

USAGE

As a starting point, for a standard 50µl 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.

ENDONUCLEASE-EXONUCLEASE(DNase)

1 µg of λ Hind 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 $MgCl_2$, 0.001%(w/v) gelatin. No degradation of the DNA fragment 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 $MgCl_2$, 0.001%(w/v) gelatin. No degradation of the DNA fragment 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 $MgCl_2$, 0.001%(w/v) gelatin.
No degradation of the tRNA was detected by polyacrylamide gel electrophoresis.

SIZE DETERMINATION

A 3.75 µg and 5 µg sample were run on a 17-27% SDS-PAGE gel for 1.5 hours at 35 mAmps in a 1X Tris-Glycine-SDS buffer. Each sample was mixed 1:1 in loading buffer containing 0.125 M Tris-HCl, pH 6.8, 4% (w/v) SDS, 20% (v/v) glycerol and 0.3%(w/v) bromophenol blue. β-Mercaptoethanol was added to the sample solution to a final concentration of 1.3 M to reduce protein. Each sample was heated at 95°C for 2 minutes and cooled on ice prior to loading onto gel.

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 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. Quin, C., Nucleic Acids Research, **20**, No. 16, 4371 1992)

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

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