Monolith Protocol MO-P-035

ssDNA – EcoSSB

EcoSSB (*E. coli* single-stranded DNA binding protein) plays a key role during DNA replication by binding single-stranded DNA (ssDNA), thereby preventing re-annealing of the strands and allowing replication by DNA primases and polymerases. Biophysical *in vitro* and crystallographic studies showed that one EcoSSB tetramer binds a single ssDNA oligonucleotide over a total length of 70 nucleotides.

protein – DNA interaction | DNA replication

A1. Target/Fluorescent Molecule

70 mer ssDNA

A2. Molecule Class/Organism

Single-stranded DNA (ssDNA)
Escherichia coli

A3. Sequence/Formula

```
5’ Cy5 TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT
       TTT TTT TTT T 3’
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A4. Purification Strategy/Source

Bio-Synthesis, Inc.

A5. Stock Concentration/Stock Buffer

10 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

21.9 kDa
577,600 $M^{-1}cm^{-1}$ (ε_{260})

A7. Dilution Buffer

10 mM HEPES, pH 7.4, 300 mM NaCl, 0.05% TWEEN® 20

A8. Labeling Strategy

5’-Cy5 labeled

A9. Labeling Procedure

N/A

A10. Labeling Efficiency

Dual HPLC-purification performed by Bio-Synthesis, 100% labeled DNA

B1. Ligand/Non-Fluorescent Binding Partner

EcoSSB
uniprot.org/uniprot/P0AGE0

B2. Molecule Class/Organism

DNA replication protein
Escherichia coli

B3. Sequence/Formula

MASRGVNKVI LVGNLGQDPE VRYMPNGGAV ANITLATSES WRDKATGEMK EQTEWHRVVL FGKLAEVASE YLRKGSQVYI
EGQLRTRKWT DQSGQDRYTT EVVVNVGGTM QMLGGRQGGG APAGGNIGGG QPQGGWGQPQ QPQGGNQFSG GAQSRPQQSA
PAAPSNEPPM DFDDDIPF

B4. Purification Strategy/Source

Recombinant E. coli SSB protein
Abcam
ab123224

B5. Stock Concentration/Stock Buffer

2 mg/mL | 105 µM
0.32% Tris HCl, pH 7.6, 1.17% NaCl, 0.03% EDTA, 0.02% DTT, 50% glycerol

B6. Molecular Weight/Extinction Coefficient

19 kDa
27,960 $M^{-1}cm^{-1}$ (ε_{280})

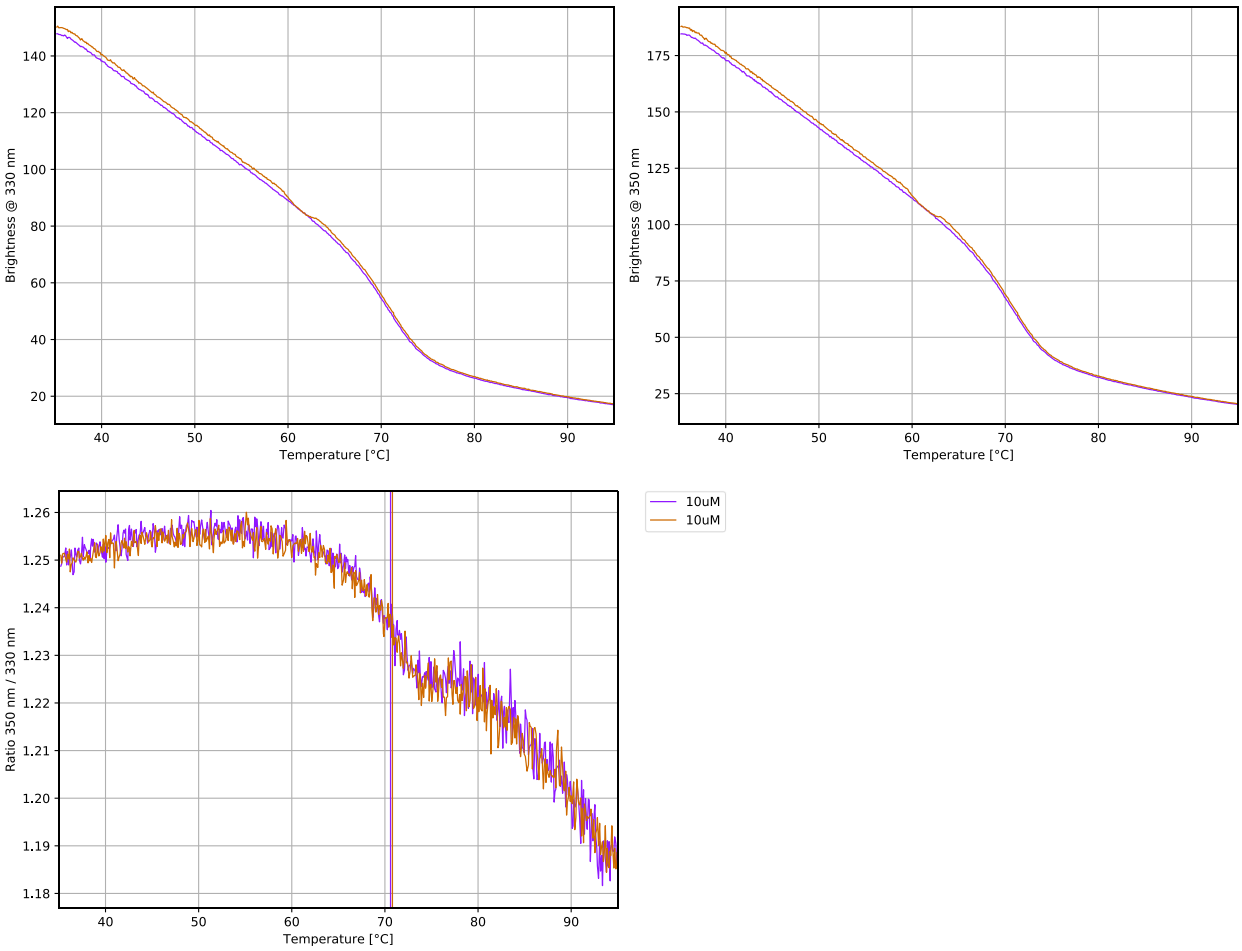
B7. Serial Dilution Preparation

1. Add 2 µL of EcoSSB (105 µM) to 198 µL of dilution buffer to obtain 200 µL of a 1.05 µM EcoSSB solution.
2. Add 2 µL of EcoSSB (1.05 µM) to 198 µL of dilution buffer to obtain 200 µL of a 10.5 nM EcoSSB solution.
3. Add 5.7 µL of EcoSSB (10.5 nM) to 24 µL of dilution buffer to obtain 29.7 µL of a 2 nM EcoSSB solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 2 nM EcoSSB solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
5. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
6. Add 2 µL of ssDNA (10 µM) to 198 µL of dilution buffer to obtain 200 µL of a 100 nM ssDNA solution.
7. Add 2 µL of ssDNA (100 nM) to 198 µL of dilution buffer to obtain 200 µL of a 1 nM ssDNA solution.
8. Add 8 µL of ssDNA (1 nM) to 192 µL of dilution buffer to obtain 200 µL of a 40 pM ssDNA solution.
9. Add 10 µL of 40 pM Cy5-labeled ssDNA to each tube from **16** to **1** and mix by pipetting.
10. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity of EcoSSB using Tycho NT.6:
nanotempertech.com/tycho

10 uM	10 µL of 10 µM EcoSSB (technical replicates)	T_i = 70.7°C ± 0.1°C



D1. MST System/Capillaries

Monolith NT.115^{PICO} Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

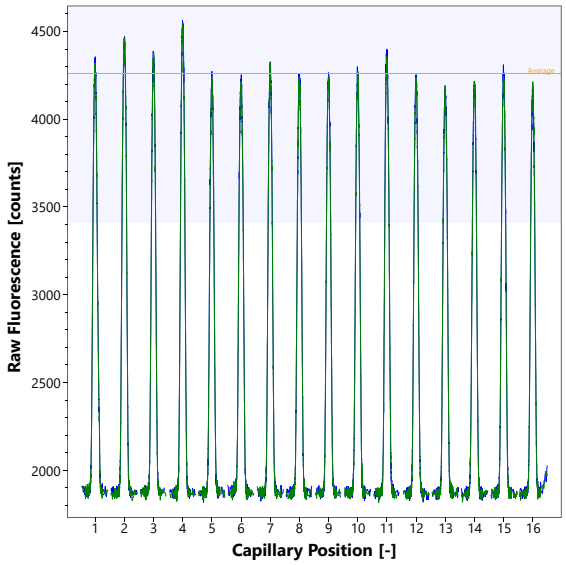
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

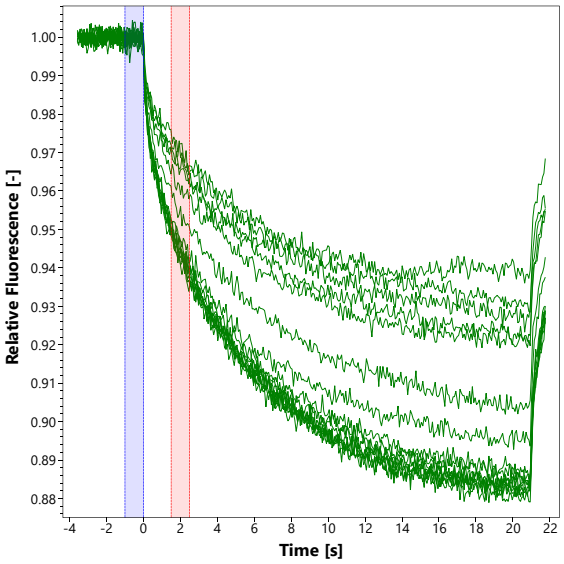
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

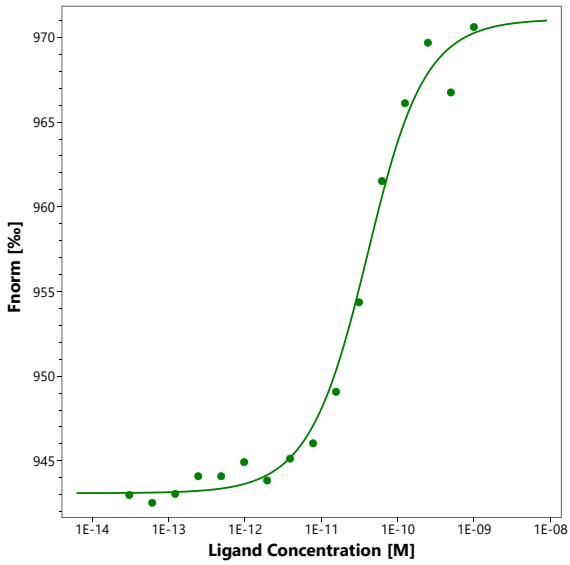
20 mM HEPES, pH 7.4, 300 mM NaCl, 0.05% TWEEN® 20
20 pM ssDNA | 1 nM – 30 fM EcoSSB | 25°C | medium MST power | 100% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 33.2 pM ± 2.9 pM (average of 3 independent replicates)







D5. Reference Results/Supporting Results

N/A

E. Contributors

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