**EcSSB G26C: information and sequence**

**Plasmid**

pET22b\_EcSSB\_1

**Usage**

The expressed protein is a single-cysteine variant of E. coli single-stranded DNA-binding protein, EcSSB, for subsequent labeling at the cysteine with one IDCC (N-[2-(iodoacetamido)ethyl]-7-diethylaminocoumarin-3-carboxamide) per subunit. This adduct is then used as a fluorescent single-stranded DNA (ssDNA) biosensor, having ~6-fold fluorescence increase on binding ssDNA with very high affinity.

**Publications**

Dillingham, M. S.; Tibbles, K. L.; Hunter, J. L.; Bell, J. C.; Kowalczykowski, S. C.; Webb, M. R. (2008), Fluorescent single-stranded DNA binding protein as a probe for sensitive, real time assays of helicase activity. Biophys. J. 95, 3330-3339. (PMID: 18599625)

Kunzelmann, S.; Morris, C.; Chavda, A. P.; Eccleston, J. F.; Webb, M. R. (2010), Mechanism of interaction between single-stranded DNA binding protein and DNA. Biochemistry 49, 843-852.

Hedgethorne, K.; Webb, M. R. (2012), Fluorescent SSB as a reagentless biosensor for single-stranded DNA. Methods Mol. Biol., 922, 219-233.

**Protocol**

See Hedgethorne and Webb (2012) for latest protocol.

**Contact**

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**Plasmid Sequence**

Ssb gene is in pET22B vector

GGGTGAACGGTACATTCCGTCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCCAGCAGAGGCGTAAACAAGGTTATTCTCGTTGGTAATCTGGGTCAGGACCCGGAAGTACGCTACATGCCAAATTGTGGCGCAGTTGCCAACATTACGCTGGCTACTTCCGAATCCTGGCGTGATAAAGCGACCGGCGAGATGAAAGAACAGACTGAATGGCACCGCGTTGTGCTGTTCGGCAAACTGGCAGAAGTGGCGAGCGAATATCTGCGTAAAGGTTCTCAGGTTTATATCGAAGGTCAGCTGCGTACCCGTAAATGGACCGATCAATCCGGTCAGGATCGCTACACCACAGAAGTCGTGGTGAACGTTGGCGGCACCATGCAGATGCTGGGTGGTCGTCAGGGTGGTGGCGCTCCGGCAGGTGGCAATATCGGTGGTGGTCAGCCGCAGGGCGGTTGGGGTCAGCCTCAGCAGCCGCAGGGTGGCAATCAGTTCAGCGGCGGCGCGCAGTCTCGCCCGCAGCAGTCCGCTCCGGCAGCGCCGTCTAACGAGCCGCCGATGGACTTTGATGATGACATTCCGTTCTGAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATTGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCATCGCCCTGATAGACGTTTTCGCCCTTTGACGTGGAGTCACGTTCTTTATAGTGACTCTGTCAAACTGACACACTCACCCTATCTCGGTCTATTCTTTGATTTATAGGATTTGCGATTCCGCTATGTTTAGATAGCTGATACCAAGTACGCGAATTACTAATTTACGCCTTACCAATTTAG

**Protein sequence**

Met A S R G V N K V I L V G N L G Q D P E V R Y Met P N C G A V A N I T L A T S E S W R D K A T G E Met K E Q T E W H R V V L F G K L A E V A S E Y L R K G S Q V Y I E G Q L R T R K W T D Q S G Q D R Y T T E V V V N V G G T Met Q Met L G G R Q G G G A P A G G N I G G G Q P Q G G W G Q P Q Q P Q G G N Q F S G G A Q S R P Q Q S A P A A P S N E P P Met D F D D D I P F Stop