**EcSSB W88C: information and sequence**

**Plasmid**

pET22b\_EcSSB\_2

**Usage**

The expressed protein is a single-cysteine variant of E. coli single-stranded DNA-binding protein, EcSSB, for subsequent labeling at the cysteine. The adduct with Cy3B maleimide has been used as a biosensor for single-stranded DNA in TIRF microscopy measurements.

**Publications**

Fili, N.; Mashanov, G.; Toseland, C. P.; Batters, C.; Wallace, M. I.; Yeeles, J. T. P.; Dillingham, M. S.; Webb, M. R.; Molloy, J. E., Visualizing DNA unwinding by helicases at the single molecule level. Nucleic Acids Res. 2010, 38, 4448-4457.

Chisty, L. T.; Toseland, C. P.; Fili, N.; Mashanov, G. I.; Dillingham, M. S.; Molloy, J. E.; Webb, M. R., Monomeric PcrA helicase processively unwinds plasmid lengths of DNA in the presence of the initiator protein RepD. Nucleic Acids Res. 2013, 41, 5010–5023.

Fili, N.; Toseland, C. P.; Dillingham, M. S.; Webb, M. R.; Molloy, J. E., A single-molecule approach to visualize DNA unwinding. Methods Mol. Biol. 2011, 778, 193-214.

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

**Protocol**

See Hedgethorne and Webb (2012) for latest protein preparation, other publications for usage.

**Contact**

Martin Webb

The Francis Crick Institute

London

mwebb2@mrw.org.uk

**Sequence EcSSB W88C**

Ssb is in pET22B vector

TGCGCCATTCGATGGTGTCCGGGATCTCGACGCTCTCCCTTATGCGACTCCTGCATTAGGAAGCAGCCCAGTAGTAGGTTGAGGCCGTTGAGCACCGCCGCCGCAAGGAATGGTGCATGCAAGGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCCAGCAGAGGCGTAAACAAGGTTATTCTCGTTGGTAATCTGGGTCAGGACCCGGAAGTACGCTACATGCCAAATGGTGGCGCAGTTGCCAACATTACGCTGGCTACTTCCGAATCCTGGCGTGATAAAGCGACCGGCGAGATGAAAGAACAGACTGAATGGCACCGCGTTGTGCTGTTCGGCAAACTGGCAGAAGTGGCGAGCGAATATCTGCGTAAAGGTTCTCAGGTTTATATCGAAGGTCAGCTGCGTACCCGTAAATGCACCGATCAATCCGGTCAGGATCGCTACACCACAGAAGTCGTGGTGAACGTTGGCGGCACCATGCAGATGCTGGGTGGTCGTCAGGGTGGTGGCGCTCCGGCAGGTGGCAATATCGGTGGTGGTCAGCCGCAGGGCGGTTGGGGTCAGCCTCAGCAGCCGCAGGGTGGCAATCAGTTCAGCGGCGGCGCGCAGTCTCGCCCGCAGCAGTCCGCTCCGGCAGCGCCGTCTAACGAGCCGCCGATGGACTTTGATGATGACATTCCGTTCTGAGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAG

**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 G 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 C 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