bei resources

Shiga Toxin Type 1 Subunit A, Recombinant from *Escherichia coli*

Catalog No. NR-859

This reagent is the tangible property of the U.S. Government.

For research use only. Not for human use.

Contributor:

Alison D. O'Brien, Ph.D., Chairperson, and James F. Sinclair, Ph.D., Laboratory Supervisor, Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Product Description:

The Shiga toxin (Stx) family refers to two types of related toxins: Shiga toxin type 1 (Stx1, Shiga-like toxin 1, or verotoxin 1) and Shiga toxin type 2 (Stx2, Shiga-like toxin 2, or verotoxin 2).¹ Stx1 is almost identical to Shiga toxin produced by Shigella dysenteriae at the nucleotide sequence level, while Stx2 shares approximately 55% overall nucleotide sequence homology with Stx1 and Shiga toxin. Shiga toxins are multimeric molecules that are comprised of two polypeptide subunits, A and B. The B subunit is a pentamer that binds the toxin to glycolipids on host cell membranes and the entire toxin molecule can then enter the cell via endocytosis.² Once inside the cell, the A subunit undergoes proteolytic cleavage and the reduction of an internal disulfide bond to generate Stx A1 and Stx A2. Stx A1 is an N-glycosidase that catalytically inactivates the 28S ribosomal RNA subunit to inhibit protein synthesis.

The sequences of the structural genes for Shiga toxin from *Shigella dysenteriae* and Shiga toxin type 2 from *E. coli* have been determined.^{4,5} The crystal structure of Shiga toxin from *Shigella dysenteriae* and Shiga toxin type 2 from *E. coli* have been solved (PDB: 1DM0 and 1R4P, respectively).^{6,7}

NR-859 is a recombinant protein of Shiga toxin type 1 subunit A (rStx1A).^{8,9} The protein sequence¹⁰ of the native Shiga toxin A subunit was modified to include an internal penta-histidine-tag used for the purpose of purification. The tagged protein was produced using a Qiagen QE30 series his-tag vector series, expressed in *E. coli*, and purified by nickel affinity chromatography. The resulting protein, NR-859, has a molecular weight of approximately 34,900 daltons. The predicted protein sequence of NR-859 is shown in Table 1 below.

Material Provided:

Each vial contains 0.1 mg of NR-859 suspended in phosphate buffered saline. The concentration, expressed as mg/mL is shown on the Certificate of Analysis.

Packaging/Storage:

NR-859 was packaged aseptically in plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Repeated freeze-thaw cycles should be avoided.

Functional Activity:

NR-859 reacts with rabbit polyclonal antiserum specific for Stx type 1 subunit A. Carbonic anhydrase negative control is not recognized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Shiga Toxin Type 1 Subunit A, Recombinant from *Escherichia coli*, NR-859."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see <u>www.cdc.gov/biosafety/publications/bmbl5/index.htm</u>.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at <u>www.beiresources.org</u>.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC[®] nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC[®] nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC[®] and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC[®], their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

BEI Resources www.beiresources.org E-mail: <u>contact@beiresources.org</u> Tel: 800-359-7370 Fax: 703-365-2898 **b**|**e**|**i** resources

SUPPORTING INFECTIOUS DISEASE RESEARCH

Use Restrictions:

This material is distributed for internal research, noncommercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale. This material may be subject to third party patent rights.

References:

- Nakao, H. and T. Takeda. "Escherichia coli Shiga Toxin." J. Nat. Toxins 9 (2000): 299-313. PubMed: 10994531.
- Sandvig, K., et al. "Endocytosis from Coated Pits of Shiga Toxin: A Glycolipid-binding Protein from Shigella dysenteriae 1." <u>J. Cell Biol.</u> 108 (1989): 1331-1343. PubMed: 2564398.
- Skinner, L. M. and M. P. Jackson. "Investigation of Ribosome Binding by the Shiga Toxin A1 Subunit, Using Competition and Site-Directed Mutagenesis." <u>J. Bacteriol.</u> 179 (1997): 1368-1374. PubMed: 9023224.
- Strockbine, N. A., et al. "Cloning and Sequencing of the Genes for Shiga Toxin from *Shigella Dysenteriae* Type 1." <u>J. Bacteriol.</u> 170 (1988): 1116-1122. PubMed: 2830229. GenPept: AAA98347 and AAA98348.

- Jackson, M. P., et al. "Nucleotide Sequence Analysis and Comparison of the Structural Genes for Shiga-Like Toxin 1 and Shiga-Like Toxin 2 Encoded by Bacteriophages from *Escherichia coli*." <u>FEMS Microbiol. Lett.</u> 44 (1987): 109-114. GenPept: CAA30714 and CAA30715.
- Fraser, M. E., et al. "Crystal Structure of the Holotoxin from Shigella dysenteriae at 2.5 Å Resolution." <u>Nat.</u> <u>Struct. Biol.</u> 1 (1994): 59-64. PubMed: 7656009. PDB: 1DM0.
- Fraser, M. E., et al. "Structure of Shiga Toxin Type 2 (Stx2) from *Escherichia coli* O157:H7." <u>J. Biol. Chem.</u> 279 (2004): 27511-27517. PubMed: 15075327. PDB: 1R4P.
- Paton, J. C. and A. W. Paton. "Pathogenesis and Diagnosis of Shiga Toxin-Producing *Escherichia coli* Infections." <u>Clin. Microbiol. Rev.</u> 11 (1998): 450–479. PubMed: 9665978.
- 9. O'Brien, A. D. and R. K. Holmes. "Shiga and Shiga-Like Toxins." <u>Microbiol. Rev.</u> 51 (1987): 206–220. PubMed: 3299029.
- Unkmeir, A. and H. Schmidt. "Structural Analysis of Phage-Borne Stx Genes and Their Flanking Sequences in Shiga Toxin-Producing *Escherichia coli* and *Shigella dysenteriae* Type 1 Strains." <u>Infect. Immun.</u> 68 (2000): 4856–4864. PubMed: 10948097. GenPept: CAC05535.

ATCC[®] is a trademark of the American Type Culture Collection.



Table 1 – Predicted Protein Sequence					
1	MKIIIFRVLT	FFFVIFSVNV	VAKEFTLDFS	TAKTYVDSLN	VIRSAIGTPL
51	QTISSGGTSL	LMIDSGSGDN	LFAVDVRGID	PEEGRFNNLR	LIVERNNLYV
101	TGFVNRTNNV	FYRFADFSHV	TFPGTTAVTL	SGDSSYTTLQ	RVAGISRTGM
151	QINRHSLTTS	YLDLMSHSGT	SLTQSVARAM	LRFVTVTAEA	LRFRQIQRGF
201	RTTLDDLSGR	SYVMTAEDVD	LTLNWGRLSS	VLPDYHGQDS	VRVGRISFGS
251	INAILGSVAL	ILNC HHHHH R	VARMASDEFP	SMCPADGRVR	GITHNKILWD
301	SSTLGAILMR	RTISS			