

Shiga Toxin Type 1, Recombinant from *Escherichia coli*

Catalog No. NR-857

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Contributor and Manufacturer:

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Product Description:

Recombinant Shiga toxin type 1 was expressed in *Escherichia coli* (*E. coli*) and purified by affinity chromatography essentially as described.^{1,2}

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).³ 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.⁴ 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.⁵

The sequences of the structural genes for Shiga toxin from *Shigella dysenteriae* and Shiga toxin type 1 from *E. coli* have been determined.^{6,7} The crystal structure of Shiga toxin from *Shigella dysenteriae* has been solved (PMB: 1DM0).⁸

The predicted protein sequences of the A and B subunits of NR-857 are shown in Tables 1 and 2 below.

Material Provided:

Each vial of NR-857 lot 70004145 contains approximately 10 µg of Shiga toxin type 1 suspended in buffer. Each vial of NR-857 lot 6176493 contains approximately 10 µg of Shiga toxin type 1 suspended in 10 mM sodium phosphate buffer (pH 5.7) and 50 mM sodium chloride. Each vial of NR-857 lots 58338341 and 60861998 contain approximately 14 µg of Shiga toxin type 1 suspended in PBS, pH 7.4. The concentration, expressed as mg/mL is shown on the Certificate of Analysis.

Packaging/Storage:

NR-857 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-857 reacts with rabbit polyclonal antiserum specific for Stx type 1 and demonstrates cytotoxicity in Vero cells.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Shiga Toxin Type 1, Recombinant from *Escherichia coli*, NR-857."

Biosafety Level: 2

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. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

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References:

1. Perera, L. P., J. E. Samuel, R. K. Holmes, and A. D. O'Brien. "Identification of Three Amino Acid Residues in the B Subunit of Shiga Toxin and Shiga-Like Toxin Type II That Are Essential for Holotoxin Activity." *J. Bacteriol.* 173 (1991): 1151-1160. PubMed: 1991714.
2. Ryd, M., et al. "Purification of Shiga Toxin by α -D-Galactose-(1 \rightarrow 4)- β -D-Galactose-(1 \rightarrow 4)- β -D-glucose-(1 \rightarrow) Receptor Ligand-Based Chromatography." *FEBS Lett.* 258 (1989): 320-322. PubMed: 2689221.
3. Nakao, H. and T. Takeda. "Escherichia coli Shiga Toxin." *J. Nat. Toxins* 9 (2000): 299-313. PubMed: 10994531.
4. Sandvig, K., et al. "Endocytosis from Coated Pits of Shiga Toxin: A Glycolipid-binding Protein from *Shigella dysenteriae* 1." *J. Cell Biol.* 108 (1989): 1331-1343. PubMed: 2564398.
5. Skinner, L. M. and M. P. Jackson. "Investigation of Ribosome Binding by the Shiga Toxin A1 Subunit, Using Competition and Site-Directed Mutagenesis." *J. Bacteriol.* 179 (1997): 1368-1374. PubMed: 9023224.
6. Strockbine, N. A., et al. "Cloning and Sequencing of the Genes for Shiga Toxin from *Shigella Dysenteriae* Type 1." *J. Bacteriol.* 170 (1988): 1116-1122. PubMed: 2830229.
7. Calderwood, S. B., et al. "Nucleotide Sequence of the Shiga-Like Toxin Genes of *Escherichia coli*." *Proc. Natl. Acad. Sci. U.S.A.* 84 (1987): 4364-4368. PubMed: 3299365.
8. Fraser, M. E., M. M. Chernaia, Y. V. Kozlov, and M. N. G. James. "Crystal Structure of the Holotoxin from *Shigella dysenteriae* at 2.5 Å Resolution." *Nat. Struct. Biol.* 1 (1994): 59-64. PubMed: 7656009. PDB: 1DM0.

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Table 1 – Predicted Protein Sequence A Subunit					
1	KEFTLDFSTA	KTYVDSLNI	RSAIGTPLQT	ISSGGTSLLM	IDSGTGDNLF
51	AVDVRGIDPE	EGRFNLRLL	VERNLYVTG	FVNRTNNVFY	RFADFSHVTF
101	PGTTAVTLTG	DSSYTTLQRV	AGISRTGMQI	NRHSLTTSYL	DLMSHSGTSL
151	TQSVARAMLR	FVTVTAEALR	FRQIQRGFRT	TLDDLGRSY	VMTAEDVDLT
201	LNWGRLLSVL	PDYHGQDSVR	VGRISFGSIN	AILGSVALIL	NCHHHASRVA
251	RMASDEFPSM	CPADGRVIR	THNKILWDSS	TLGAILMRRT	I

Signal sequence has been removed.

Table 2 – Predicted Protein Sequence B Subunit					
1	TPDCVTGKVE	YTKYNDDDTF	TVKVGDKELF	TNRWNLQSL	LSAQITGMTV
51	TIKTNACHNG	GGFSEVIFR			

Signal sequence has been removed.