

Shiga Toxin Type 1 Toxoid, Recombinant from *Escherichia coli*

Catalog No. NR-858

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Contributor and Manufacturer:

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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.³

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

NR-858 is a recombinant toxoid of Shiga toxin type 1 (Stx1) with genetic mutations in the catalytic A subunit which render the protein non-toxic. The recombinant A subunit includes an N-terminal hexa-histidine tag. The recombinant B subunit contains a C-terminal hexa-histidine tag. The recombinant protein was expressed in *Escherichia coli* and purified by nickel affinity chromatography. NR-858 has a theoretical molecular weight of approximately 33534 daltons for subunit A and 8513 daltons for subunit B. The predicted amino acid sequence of NR-858 is shown below in Table 1 (Subunit A) and Table 2 (Subunit B).

Material Provided:

Each vial of NR-858 contains approximately 50 µg of recombinant Stx1 toxoid suspended in phosphate buffered saline (pH 7.4). The concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

Packaging/Storage:

NR-858 was packaged aseptically in plastic cryovials. The

product is provided frozen on dry ice and should be stored at -70°C or colder immediately upon arrival. **Note: The label for lot 57680169 incorrectly indicates that it should be stored at -20°C or colder.** Repeated freeze-thaw cycles should be avoided.

Functional Activity:

NR-858 reacts with rabbit polyclonal antibody⁷ to Shiga toxin type 1 and is not toxic to Vero cells.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: Shiga Toxin Type 1 Toxoid, Recombinant from *Escherichia coli*, NR-858."

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. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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References:

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5. 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. GenPept: AAA98099 and AAA98100.
6. Fraser, M. E., et al. "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 for Stx1 Subunit A					
1	<u>MRGSHHHHHH</u>	<u>GS</u> KFTLDFS	TAKTYVDSL	VIRSAIGTPL	QTISSGGTSL
51	LMIDSGSGDN	LFAVDV	RGID	PEEGRFNNLR	LIVERNNLS*V
101	FYRFADFSHV	TFPGTTAVTL	SGDSSYTTLQ	RVAGISRTGM	QINRHSLTTS
151	YLDLMSHSGT	SLTQSVARAM	LRFVTVTAQ*A	LRFRQIQRGF	RTTLDDLGR
201	SYVMTAEDVD	LTLNWGRLSS	VLPDYHGQDS	VRVGRISFGS	INAILGSVAL
251	ILNCHHHASR	VARMASDEFP	SMCPADGRVR	GITHNKILWD	SSTLGAILMR
301	R T I S S				

*Mutagenized catalytic residues Y89S and E179Q (based on the recombinant sequence above).
Non-shiga toxin residues are underlined. The recombinant protein does not contain signal peptide residues.

Table 2 – Predicted Protein Sequence for Stx1 Subunit B					
1	TPDCVTGKVE	YTKYNDDDTF	TVKVGDKELF	TNRWNLQSL	LSAQITGMTV
51	TIKTNACHNG	GGFSEVIFRH	<u>HHHHH</u>		

Non-shiga toxin residues are underlined. The recombinant protein does not contain signal peptide residues.