

## Shiga Toxin Type 2 Toxoid, Chemically Inactivated

### Catalog No. NR-4675

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### For research use only. Not for human use.

#### Contributor and Manufacturer:

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#### Product Description:

Recombinant Shiga toxin Type 2 (Stx2) was expressed in *Escherichia coli*, purified by affinity chromatography, and chemically inactivated with formaldehyde. The recombinant toxin has a theoretical molecular weight of approximately 33194 daltons for subunit A and 7817 daltons for subunit B. The predicted amino acid sequence is shown below in Table 1 (Subunit A) and Table 2 (Subunit B).

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).<sup>1</sup> 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.<sup>2</sup> Once inside the cell, the A subunit undergoes proteolytic cleavage and the reduction of an internal disulfide bond to generate Stx A<sub>1</sub> and Stx A<sub>2</sub>. Stx A<sub>1</sub> is an N-glycosidase that catalytically inactivates the 28S ribosomal RNA subunit to inhibit protein synthesis.<sup>3</sup>

The sequences of the structural genes for Shiga toxin from *Shigella dysenteriae* and Stx2 from *E. coli* have been determined.<sup>4</sup> The crystal structure of Stx2 has been solved (PDB: 1R4P).<sup>5</sup>

#### Material Provided:

Each vial of NR-4675 contains approximately 8 µg of chemically inactivated Stx2 toxoid suspended in PBS (pH 7.4). The protein content and concentration, expressed as mg per mL, are shown on the Certificate of Analysis.

#### Packaging/Storage:

NR-4675 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. Repeated freeze-thaw cycles should be avoided.

#### Functional Activity:

NR-4675 reacts with rabbit polyclonal antibody to Stx2 and is not cytotoxic to Vero cells at concentrations over 3 logs greater than the CD<sub>50</sub> of recombinant Stx2.

#### Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Shiga Toxin Type 2 Toxoid, Chemically Inactivated, NR-4675."

#### 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, 2009; see [www.cdc.gov/biosafety/publications/bmbl5/index.htm](http://www.cdc.gov/biosafety/publications/bmbl5/index.htm).

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**References:**

1. Nakao, H. and T. Takeda. "Escherichia coli Shiga Toxin." *J. Nat. Toxins* 9 (2000): 299-313. PubMed: 10994531.
2. 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.
3. 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.
4. 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.
5. Fraser, M. E., et al. "Structure of Shiga Toxin Type 2 (Stx2) from *Escherichia coli* O157:H7." *J. Biol. Chem.* 279 (2004): 27511-27517.

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**Table 1 – Predicted Protein Sequence for Stx2 Subunit A**

1	REFTIDFSTQ	QSYVSSLNSI	RTEISTPLEH	ISQGTTSVSV	INHTPPGSYF
51	AVDIRGLDVY	QARFDHLRLI	IEQNNLYVAG	FVNTATNTFY	RFSDFTHISV
101	PGVTTVSMTT	DSSYTTLQRV	AALERSGMQI	SRHSLVSSYL	ALMEFSGNTM
151	TRDASRAVLR	FVTVTAEALR	FRQIQREFRQ	ALSETAPVYT	MTPGDVDLTL
201	NWGRISNVLP	EYRGEDGVRV	GRISFNNISA	ILGTVAVILN	CHHQGARSVR
251	AVNEESQPEC	QITGDRPVIK	INNTLWESNT	AAFLNRKSQ	FLYTTGK

**Table 2 – Predicted Protein Sequence for Stx2 Subunit B**

1	ADCAKGKIEF	SKYNEDDTFT	VKVDGKEYWT	SRWNLQPLLQ	SAQLTGMTVT
51	IKSSTCESGS	GFAEVQFNND			