Shiga Toxin Type 2 Toxoid, Recombinant from *Escherichia coli*

Catalog No. NR-4676

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Contributor and Manufacturer:

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Product Description:

NR-4676 is a recombinant toxoid of Shiga toxin type 2 (Stx2) with genetic mutations in the catalytic A subunit which render the protein non-toxic. The recombinant B subunit includes a C-terminal hexa-histidine tag. The recombinant toxoid was expressed in *Escherichia coli* (*E. coli*) and purified by nickel affinity chromatography. NR-4676 has a theoretical molecular weight of approximately 33080 daltons for subunit A and 8640 daltons for subunit B. The predicted amino acid sequence of NR-4676 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).[1] Stx1 is almost identical to Shiga toxin produced by *Shigella dysenteriae* (*S. 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 A_1 and Stx A_2. Stx A_1 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 *S. dysenteriae* and Shiga toxin type 2 from *E. coli* have been determined.[4,5] The crystal structure of Shiga toxin from *S. dysenteriae* and Shiga toxin type 2 from *E. coli* have been solved (PDB: 1DM0 and 1R4P, respectively).[6,7]

Material Provided:

Each vial of NR-4676 contains approximately 50 µg of recombinant Stx2 toxoid suspended in phosphate buffered saline. The concentration, expressed as mg per mL, is shown on the Certificate of Analysis.

Packaging/Storage:

NR-4676 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-4676 reacts with rabbit polyclonal antibody to Stx2 and is not cytotoxic in Vero cells.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Shiga Toxin Type 2 Toxoid, Recombinant from *Escherichia coli*, NR-4676."

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.

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References:

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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.
7. Fraser, M. E., et al. "Structure of Shiga Toxin Type 2 (Stx2) from Escherichia coli O157:H7." J. Biol. Chem. 279 (2004): 27511-27517. PubMed: 15075327.

Support Provided by NIAID

Table 1 – Predicted Protein Sequence for Stx2 Subunit A					
1	REFTIDFSTQ	QSYVSSLNSI	RTEISTPLEH	ISQGTTSVSV	INHTPPGSYF
51	AVDIRGLDVY	QARFDHLRLI	IEQNNL**S***VAG	FVNTATNTFY	RFSDFTHISV
101	PGVTTVSMTT	DSSYTTLQRV	AALERSGMQI	SRHSLVSSYL	ALMEFSGNTM
151	TRDASRAVLR	FVTVTA**Q***AL**L***	FRQIQREFRQ	ALSETAPVYT	MTPGDVDLTL
201	NWGRISNVLP	EYRGEDGVRV	GRISFNNISA	ILGTVAVILN	CHHQGARSVR
251	AVNEESQPEC	QITGDRPVIK	INNTLWESNT	AAAFLNRKSQ	FLYTTGK

*Mutagenized catalytic residues Y77S, E167Q and R170L. The recombinant protein does not contain signal peptide residues.

Table 2 – Predicted Protein Sequence for Stx2 Subunit B					
1	ADCAKGKIEF	SKYNEDDTFT	VKVDGKEYWT	SRWNLQPLLQ	SAQLTGMTVT
51	IKSSTCESGS	GFAEVQFNND	HHHHHH		

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

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