

## **Certificate of Analysis for NR-4677**

## Shiga Toxin Type 2 Subunit B, Recombinant from Escherichia coli

## Catalog No. NR-4677

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

**Product Description:** NR-4677 is a recombinant form of the B subunit of Shiga toxin type 2 (Stx2). The amino acid sequence includes a C-terminal hexa-histidine tag and amino acid residues 20 to 89 of the Stx2 subunit B protein (GenPept: AAD25446). The recombinant protein was expressed in *Escherichia coli* and purified by nickel affinity chromatography.

Lot: 57680189 Manufacturing Date: 01NOV2007

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless, no particulate matter	Clear and colorless, no particulate matter
SDS-PAGE (SYPRO Orange densitometer scan)	Dominant band of expected size (8.6 kDa) ~ 95% pure	Dominant band of ~ 8 kDa ~ 93% pure (Figure 1) <sup>1</sup>
Mass Spectrometry	Measured mass within 5% of expected mass based on amino acid sequence: 8,640 daltons	Measured mass within 0.1% of expected mass based on amino acid sequence: 8,640 daltons
SELDI-TOF Mass Spectrometry of Lys-C Digest	> 50% of total residues accounted for in peptides of expected mass	79% of total residues accounted for in peptides of expected mass
Concentration by Bicinchoninic Acid Protein Assay	Report results	1.02 mg/mL
Functional Activity by Western Blot Analysis Rabbit polyclonal antibody to Stx2 subunit B (BEI Resources NR-9352) Carbonic anhydrase	Reactive Non-reactive	Reactive (Figure 2)  Non-reactive
Cytotoxicity in Vero Cells NR-4677 Stx2 recombinant holotoxin	Report results Report results	Non-cytotoxic at 1x10 <sup>-7</sup> M CD <sub>50</sub> ~ 1x10 <sup>-10</sup> M
Sterility	0.22 µm filter sterilized	0.22 µm filter sterilized
Endotoxin Content (Limulus Amoebocyte Lysate assay)	Report results	1,250 EU/mg <sup>2</sup>
Absorbance Ratio (OD <sub>280</sub> /OD <sub>260</sub> )	Report results	1.53

<sup>&</sup>lt;sup>1</sup>Bands of higher molecular weight may be multimers.

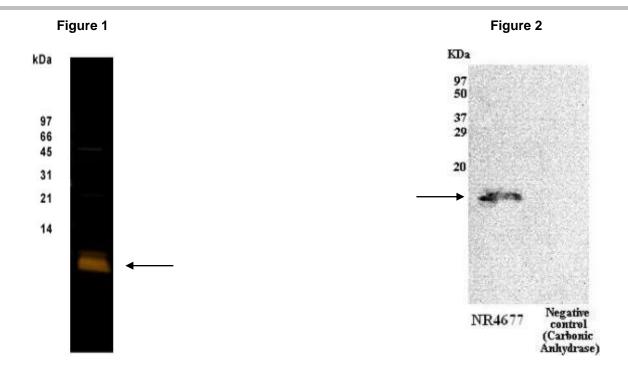
Fax: 703-365-2898

800-359-7370

<sup>&</sup>lt;sup>2</sup>NR-4677 is unsuitable for injection into animals.



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**Date:** 04 FEB 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

800-359-7370

Fax: 703-365-2898

NR-4677\_57680189\_04FEB2008

E-mail: contact@beiresources.org

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