

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

**Catalog No. NR-4676**

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

**Product Description:** NR-4676 is a recombinant toxoid of Shiga toxin type 2 (Stx2) with genetic mutations (Y77S, E167Q and R170L; based on the recombinant sequence) in the catalytic A subunit which render the protein non-toxic. The recombinant protein was expressed in *Escherichia coli* and purified by nickel affinity chromatography.

**Lot: 63487426**

**Manufacturing Date: 16FEB2016**

TEST	SPECIFICATIONS	RESULTS
<b>Appearance</b>	Clear and colorless, no particulate matter	Clear and colorless, no particulate matter
<b>SDS-PAGE (SYPRO Orange densitometry) (Figure 1)</b>	Dominant bands of expected size: Stx2A: 33.1 kDa Stx2B: 8.6 kDa	Dominant bands of expected size: Stx2A: ~ 33 kDa Stx2B: ~ 8 kDa
<b>SELDI-TOF Mass Spectrometry</b>	Measured mass within 5% of expected mass based on amino acid sequence: Stx2A: 33074 Da Stx2B: 8640 Da	Measured mass within ~3% of expected mass based on amino acid sequence: Stx2A: 33394 Da = 3.14% Stx2B: 8596 Da = 0.3%
<b>SELDI-TOF Mass Spectrometry of Trypsin Digest</b>	> 50% of total residues accounted for in peptides of expected mass	Stx2A: 56% of total residues accounted for in peptides of expected mass Stx2B: Resistant to trypsin digestion
<b>Concentration by Bicinchoninic Acid Protein Assay</b>	0.05 mg/mL ± 5%	0.05 mg/mL
<b>Functional Activity by Western Blot Analysis<sup>2</sup> (Figure 2)</b> Stx2 toxoid (NR-4676) Carbonic anhydrase	Reactive Non-reactive	Reactive Non-reactive
<b>Cytotoxicity in Vero Cells (48 hours) (Figure 3)</b> Stx2 toxoid (NR-4676) Stx2	≥ 10 <sup>4</sup> or more less toxic than holotoxin Report results	Non-cytotoxic at 1 x 10 <sup>-7</sup> M CD <sub>50</sub> ~ 1 x 10 <sup>-14</sup> M
<b>Sterility</b>	0.22 µm filter-sterilized	0.22 µm filter-sterilized
<b>Endotoxin Content (Limulus Amoebocyte Lysate assay)</b>	Report results	≤ 1250 EU per mg protein

<sup>1</sup>This item was manufactured and subjected to quality control testing by Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

<sup>2</sup>Rabbit polyclonal antibody to Stx2

**Date:** 11 MAY 2016

**Signature:**



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Figure 1: SDS-PAGE

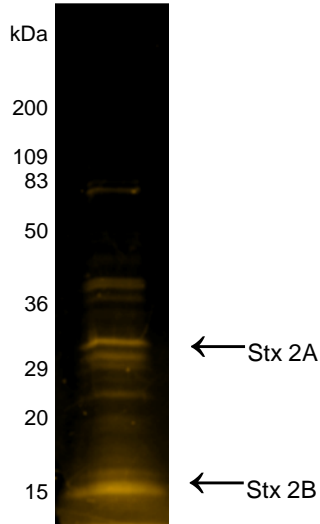
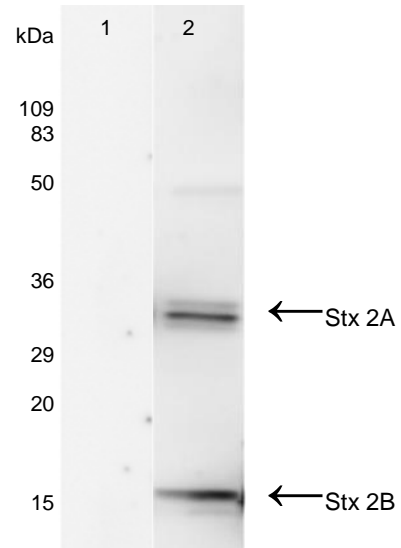


Figure 2: Western Blot



Lane 1: Carbonic anhydrase (negative control)

Lane 2: NR-4676

Figure 3: Cytotoxicity Assay

