

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

**Catalog No. NR-4478**

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

**Product Description:** Recombinant Shiga toxin type 2 [Stx2; composed of subunits A (Stx2A) and B (Stx2B)] was expressed in *Escherichia coli* and purified by affinity chromatography.

**Lot<sup>1</sup>: 58044918**

**Manufacturing Date: 15JUN2011**

TEST	SPECIFICATIONS	RESULTS
<b>Appearance</b>	Clear and colorless	Clear and colorless
<b>DNA Sequence Analysis of Vector</b>	Identical to Stx2 operon	Identical to Stx2 operon
<b>SDS-PAGE (SYPRO Orange densitometer scan)</b>	Protein bands of interest represent > 95% of total staining intensity above background: Stx2A ~ 33kDa (Stx2A <sub>1</sub> ~ 27.5 kDa and Stx2A <sub>2</sub> ~ 4.5 kDa) and Stx2B ~ 7.8 kDa	Protein bands of interest represent > 95% of total staining intensity above background: Stx2A ~ 33kDa (Stx2A <sub>1</sub> ~ 27.5 kDa and Stx2A <sub>2</sub> ~ 4.5 kDa) and Stx2B ~ 7.8 kDa (Figure 1)
<b>Mass Spectrometry</b>	Measured mass within 5% of expected mass: Stx2A: 33.2 kDa Stx2B: 7.8 kDa	Measured mass within 1% of expected mass: Stx2A: 33.4 kDa, 0.2% difference Stx2B: 7.7 kDa, 1.0% difference
<b>SELDI-TOF Mass Spectrometry of Trypsin Digest<sup>2</sup></b>	> 50% of total residues accounted for in peptides of expected mass	80% of total residues accounted for in peptides of expected (Stx2A)
<b>Concentration by Bicinchoninic Acid Protein Assay</b>	Report results	0.055 mg/mL
<b>Functional Activity by Western Blot Analysis (Figure 2)<sup>3</sup></b> Shiga toxin type 2 (NR-4478) Carbonic anhydrase (negative control)	Reactive Not reactive	Reactive (Figure 2) Not reactive (Figure 2)
<b>Cytotoxicity</b> 50% cytotoxic dose in Vero cells	Report results	5 × 10 <sup>-14</sup> M (Figure 3)
<b>Sterility</b>	0.22 µm filter sterilized	0.22 µm filter sterilized
<b>Endotoxin Content (Limulus Amoebocyte Lysate Assay)</b>	Report results	< 12.5 EU/mL

<sup>1</sup>Manufactured by Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

<sup>2</sup>Stx2B was resistant to trypsin digestion.

<sup>3</sup>Using polyclonal antiserum specific for Stx2

Figure 1 – SDS-PAGE

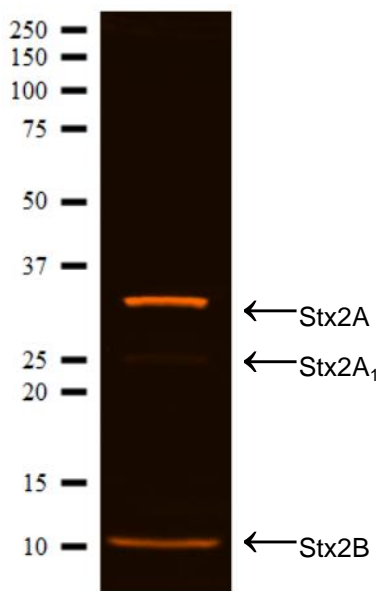
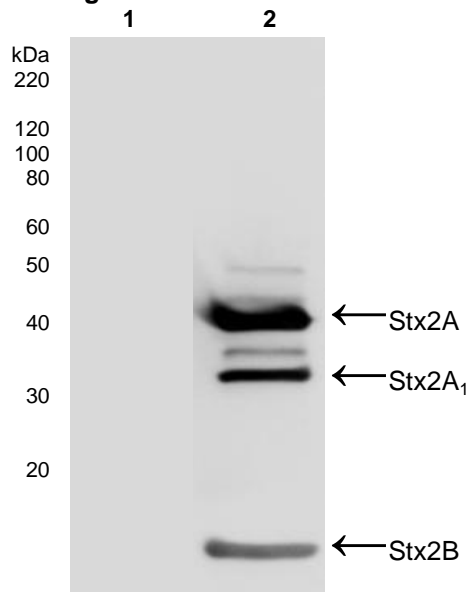
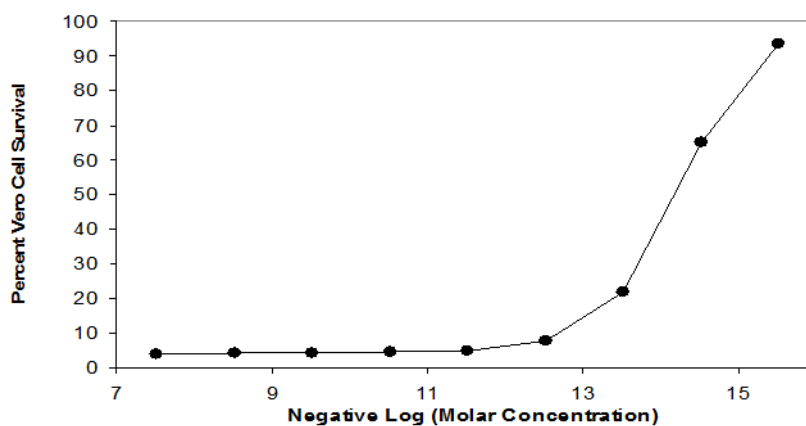


Figure 2 – Western Blot



Lane 1: Carbonic anhydrase (negative control)  
Lane 2: NR-4478 (1 µg)

Figure 3 – Cytotoxicity Assay



Date: 10 APR 2015

Signature:

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