

## **Certificate of Analysis for NR-4478**

## 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 antibody affinity chromatography.

Lot<sup>1</sup>: 62905537 Manufacturing Date: 24SEP2014

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless, no particulate matter	Clear and colorless, no particulate matter
SDS-PAGE (SYPRO Orange Densitometer Scan)	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 ~ 33 kDa (Stx2A <sub>1</sub> ~ 27.5 kDa and Stx2A <sub>2</sub> ~ 4.5 kDa) and Stx2B ~ 7.8 kDa (Figure 1)
Mass Spectrometry	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, 0.6% difference Stx2B: 7.9 kDa, 0.7% difference
SELDI-TOF Mass Spectrometry of Trypsin Digest	> 50% of total residues accounted for in peptides of expected mass	> 50% of total residues accounted for in peptides of expected mass: Stx2A: 52% of total residues Stx2B: 68% of total residues
Concentration by Bicinchoninic Acid Protein Assay	0.05 mg/mL ± 0.0025 mg/mL	0.05 mg/mL
Functional Activity by Western Blot Analysis <sup>2</sup> Shiga toxin type 2 (NR-4478) Carbonic anhydrase (negative control)	Reactive Not reactive	Reactive (Figure 2) Not reactive (Figure 2)
Cytotoxicity 50% cytotoxic dose in Vero cells	Report results	6.9 × 10 <sup>-14</sup> M (Figure 3)
Sterility	0.22 µm filter sterilized	0.22 µm filter sterilized
Endotoxin Content (Limulus Amoebocyte Lysate Assay)	Report results	< 12.5 EU/mL

Manufactured by Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA

<sup>2</sup>Using polyclonal antiserum specific for Stx2

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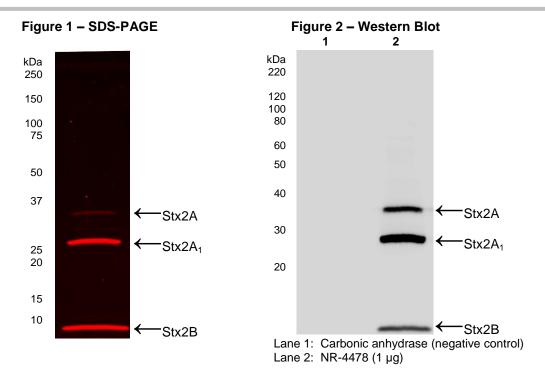
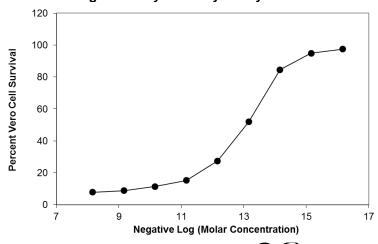


Figure 3 - Cytotoxicity Assay



**Date:** 10 APR 2015

Signature: Additional

**BEI Resources Authentication** 

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