

## **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>: 63732536 Manufacturing Date: 20OCT2015

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)
SELDI-TOF Mass Spectrometry	Measured mass within 5% of expected mass: Stx2A: 33194 Da Stx2B: 7818 Da	Measured mass within 1% of expected mass: Stx2A: 33173 Da, 0.1% difference Stx2B: 7823 Da, 0.1% 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: 63% of total residues Stx2B: 69% of total residues
Concentration by Bicinchoninic Acid Protein Assay <sup>2</sup>	0.05 mg/mL ± 0.0025 mg/mL	0.05 mg/mL
Absorbance Ratio (OD <sub>280</sub> /OD <sub>260</sub> )	Report results	Sample to dilute for analysis
Functional Activity by Western Blot Analysis³ Shiga toxin type 2 (NR-4478) Carbonic anhydrase (negative control)	Reactive Not reactive	Reactive (Figure 2) Not reactive (Figure 2)
Cytotoxicity in Vero Cells	Report results	6.9 × 10 <sup>-13</sup> M
Sterility	0.22 µm filter-sterilized	0.22 µm filter-sterilized
Endotoxin Content (Limulus Amoebocyte Lysate Assay)	Report results	< 125 EU/mL

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

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

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<sup>&</sup>lt;sup>2</sup>Performed with Pierce BCA Protein Assay Kit™ and BSA standard curve



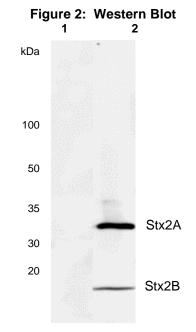
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Figure 1: SDS-PAGE

kDa

200

109
83
50
36
Stx2A
29
20
15
Stx2B



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

**Date:** 25 NOV 2015

Signature: (

**BEI Resources Authentication** 

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