b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

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 affinity chromatography.

Lot[1,2]: 57680181 **Manufacturing Date: 01DEC2007**

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 > 90% of total staining intensity above background: Stx2A ~ 33kDa ($Stx2A_1$ ~ 27.5 kDa and $Stx2A_2$ ~ 4.5 kDa) and Stx2B ~ 7.8 kDa	Protein bands of interest represent > 90% of total staining intensity above background: Stx2A ~ 33 kDa ($Stx2A_1$ ~ 27.5 kDa and $Stx2A_2$ ~ 4.5 kDa) and Stx2B ~ 7.8 kDa (Figure 1)
Mass Spectrometry	Measured mass within 5% of expected mass: Stx2A: 33,194 daltons Stx2B: 7,818 daltons	Measured mass within 1% of expected mass: Stx2A: 33,201 daltons Stx2B: 7,816 daltons
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: 80% of total residues Stx2B: 100% of total residues
Concentration by Bicinchoninic Acid Protein Assay	Report results	0.055 mg/mL
Functional Activity by Western Blot Analysis[3] Shiga toxin type 2 (NR-4478) Carbonic anhydrase (negative control)	 Reactive Not reactive	 Reactive Not reactive
Cytotoxicity 50% cytotoxic dose in Vero cells	 Report results	 6.9×10^{-12} M (Figure 2)
Sterility	0.22 µm filter sterilized	0.22 µm filter sterilized

[1]Manufactured by Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA
[2]NR-4478 was not tested for endotoxin activity due to the inherent toxicity of the protein itself. The toxicity of Shiga toxin is so much greater than that of any contaminating endotoxin that any contribution by endotoxin to the overall toxicity is minor.
[3]Using a mixture of monoclonal antibody to Stx2A (BEI Resources NR-846) and rabbit polyclonal antibody to Stx2B (BEI Resources NR-9352)

BEI Resources
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Figure 1 – SDS-PAGE

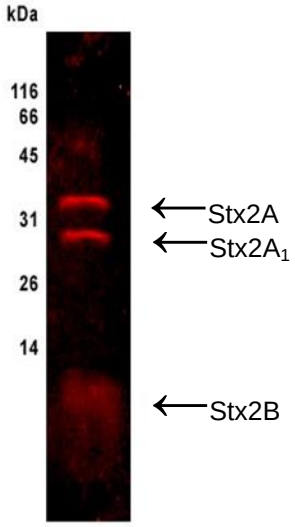
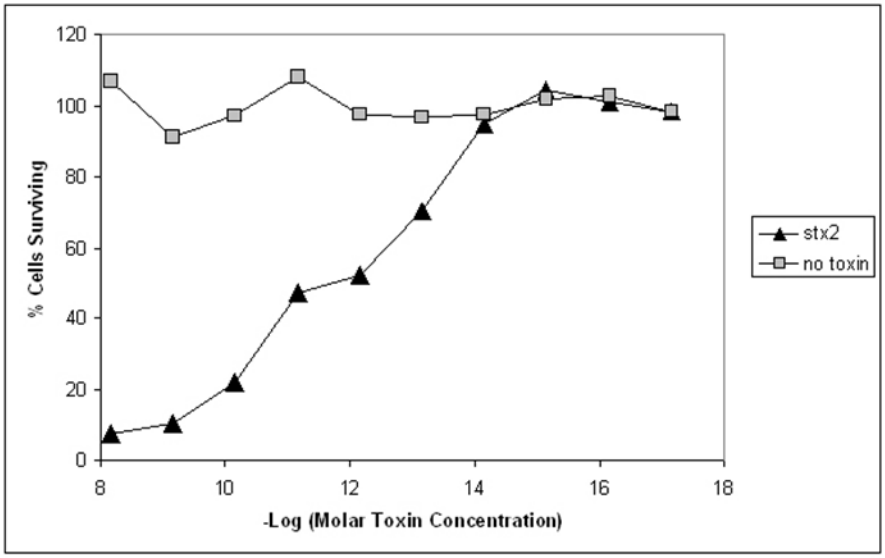


Figure 2 – Cytotoxicity Assay



Date: 10 APR 2015 **Signature:**

BEI Resources Authentication

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