

## **Certificate of Analysis for NR-858**

## Shiga Toxin Type 1 Toxoid, Recombinant from Escherichia coli

## Catalog No. NR-858

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

**Product Description:** NR-858 is a recombinant toxoid of Shiga toxin 1 (Stx1) with genetic mutations (Y89S, E179Q; 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: 57680169 Manufacturing Date: 01MAY2008

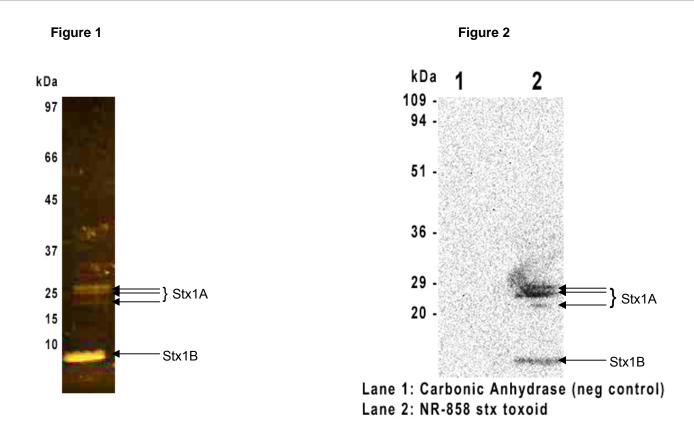
TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless, no particulate matter	Clear and colorless, no particulate matter
SDS-PAGE (SYPRO Orange densitometer scan)	Report results	Dominant bands (Figure 1): Stx1A: <sup>1</sup> ~ 30, 24 and 21 kDa Stx1B: ~ 8 kDa
Mass Spectrometry	Report results Expected mass: Stx1A: 33534 Da Stx1B: 8513 Da	Measured mass: Stx1A: <sup>1</sup> 29804, 23470 and 20609 Da Stx1B: 8529 Da
SELDI-TOF Mass Spectrometry of Trypsin Digest	> 50% of total residues accounted for in peptides of expected mass	Stx1A: 65% of total residues accounted for in peptides of expected mass Stx1B: Resistant to trypsin digestion
Concentration by Bicinchoninic Acid Protein Assay	Report results	0.48 mg/mL
Functional Activity by Western Blot Analysis <sup>1</sup> Rabbit polyclonal antibody to Stx1 Carbonic anhydrase	Reactive Non-reactive	Reactive (Figure 2) Non-reactive
Cytotoxicity in Vero Cells (48 hours) NR-858 NR-857 (Shiga Toxin Type 1, Recombinant)	Report results Report results	Non-cytotoxic at $1x10^{-7}$ M $CD_{50} \sim 1x10^{-14}$ M
Sterility	0.22 µm filter sterilized	0.22 µm filter sterilized
Endotoxin Content (Limulus Amoebocyte Lysate assay)	Report results	≤ 100 EU/mg

<sup>&</sup>lt;sup>1</sup>The A subunit of Stx1 is degraded to several slightly smaller bands/sizes than expected. This should not interfere with its use as a negative control for assaying Shiga toxin activity or as an antigen for antibody development.

800-359-7370



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**Date:** 08 SEP 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

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Fax: 703-365-2898

E-mail: contact@beiresources.org

800-359-7370