

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: 59206191 Manufacturing Date: 28JAN2011

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: ¹ ~ 34, 24 and 22 kDa Stx1B: ~ 8 kDa
Mass Spectrometry	Report results Expected mass: Stx1A: 33534 Da Stx1B: 8513 Da	Measured mass: Stx1A: 33573 Da Stx1B: 8502 Da
SELDI-TOF Mass Spectrometry of Trypsin Digest	> 50% of total residues accounted for in peptides of expected mass	Stx1A: 66% of total residues accounted for in peptides of expected mass Stx1B: 61% of total residues accounted for in peptides of expected mass
Concentration by Bicinchoninic Acid Protein Assay	Report results	0.50 mg/mL
Functional Activity by Western Blot Analysis ¹ 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 ⁻⁸ M CD ₅₀ ~ 1x10 ⁻¹³ M
Sterility	0.22 µm filter sterilized	0.22 µm filter sterilized
Endotoxin Content (Limulus Amoebocyte Lysate assay)	Report results	≤ 1.25 EU/mg

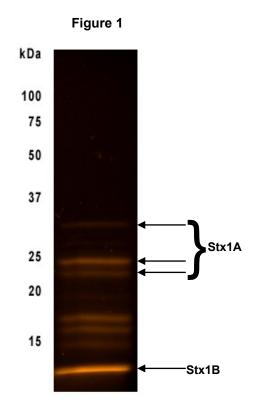
¹The A subunit of Stx1 is degraded to several slightly smaller bands/sizes than expected. This should not interfere with its use as a negative control for assaying Shiga toxin activity or as an antigen for antibody development.

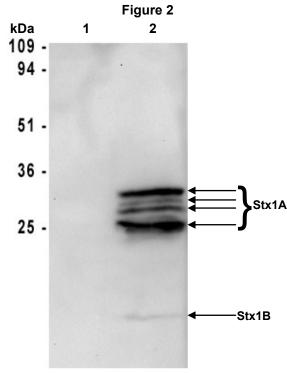
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Lane 1: Carbonic anhydrase (negative control) Lane 2: NR-858 Shiga toxin type 1 toxoid

Date: 23 MAR 2011

Signature: ∑ഗര ←

Title: Technical Manager, BEI Authentication or designee

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