b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-4477

Ricin Toxoid, Recombinant with N-Terminal Histidine Tag

Catalog No. NR-4477

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

Product Description: NR-4477 is a genetically inactivated ricin toxoid. Catalytic Glu and Arg residues in the enzyme active site have been mutagenized to create a non-toxic protein. The protein is expressed recombinantly as a single 62 kDa polypeptide containing both the A and B subunits. It also has an additional amino-terminal histidine-tag sequence. This toxoid substance is prone to aggregation. The material precipitates at neutral pH but remains soluble in basic buffer of pH 8 or higher. The protein migrates through SDS-PAGE gel as a mixture of monomer, dimer, trimer and higher order species.

Lot: 57680178 **Manufacturing Date: 14JUN2012**

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless	Clear and colorless
SDS-PAGE (SYPRO Orange Densitometer Scan)	Protein bands of interest represent > 95% of total staining intensity above background	Toxoid monomer and multimers represent > 98% of total staining intensity above background (Figure 1)
SELDI-TOF Mass Spectrometry	Measured mass is within 5% of theoretical mass (61782 Da)	Measured mass of 61752 Da is within 0.05% of theoretical mass
SELDI-TOF Mass Spectrometry of Trypsin Digest	> 50% of total residues accounted for in peptides of expected mass	57% of total residues accounted for in peptides of expected mass
Concentration by Bicinchoninic Acid[1]	Report results	1.0 mg/mL
Functional Activity **Western Blot (Figure 2)**[2] NR-4477 Carbonic anhydrase **Cytotoxicity in Vero Cells**[3] NR-4477 Active ricin toxin	 Reactive Non-reactive Report results Report results	 Reactive Non-reactive Non-cytotoxic at 1×10^{-6} M $CD_{50} \sim 1 \times 10^{-11}$ M
Sterility	0.22 µm filter-sterilized	0.22 µm filter-sterilized
Absorbance Ratio (OD_{280}/OD_{260})	Report results	1.6
Endotoxin Content (Limulus Amoebocyte Lysate Assay)	< 10,000 EU/mg	< 250 EU/mg

[1]BSA standard curve
[2]Using mouse monoclonal antibody to ricin toxin A chain (BEI Resources NR-843)
[3]Determined by the number of cells that survive 48 hours after challenge

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Figure 1 – SDS-PAGE

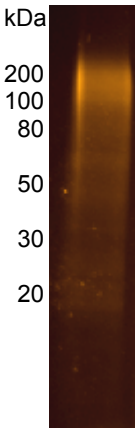
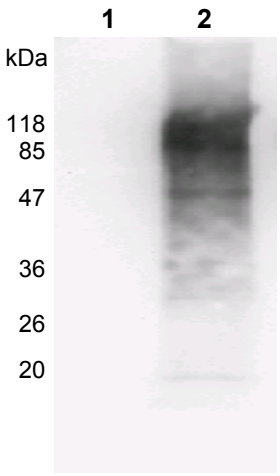


Figure 2 – Western Blot



Lane 1: Carbonic Anhydrase
Lane 2: NR-4477

Date: 20 NOV 2014

Signature:

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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