b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-4671

Ricin Toxoid, Chemically Inactivated from *Ricinus communis*

Catalog No. NR-4671

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

Product Description: Ricin toxoid was generated by chemical reduction and acylation of cysteine residue side chains of the ricin holotoxin.

Lot: 61632757 **Manufacturing Date: 10APR2013**

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless	Clear and colorless
SDS-PAGE (SYPRO Orange Densitometer Scan)	Protein band of interest represents >95% of total staining intensity above background	Ricin toxoid (multiple bands)[1] represents > 95% of total staining (Figure 1)
SELDI-TOF Mass Spectrometry	Measured value within 5% of theoretical value	A-chain measured: 32490 daltons[2] Theoretical: 30492 daltons Difference: 6.1%[2] B-chain Measured: 31261 daltons Theoretical: 29735 daltons Difference: 4.9%
SELDI-TOF Mass Spectrometry of Trypsin Digest[3]	> 50% of total residues accounted for in peptides of expected mass	62% of total residues accounted for in peptides of expected mass
Concentration by BCA Assay[4]	1.0 mg/mL ± 5%	1.0 mg/mL
Functional Activity Western blot (Figure 2)[1,5] NR-4671 Carbonic anhydrase	 Reactive Non-reactive	 Reactive Non-reactive
Cytotoxicity in Vero cells (Figure 3)[6] NR-4671 Ricin holotoxin	 Report results Report results	 Non-cytotoxic ≤ 3×10^{-7} M $CD_{50} \sim 3 \times 10^{-11}$ M
Sterility	0.22 µm filter-sterilized	0.22 µm filter-sterilized
Absorbance Ratio (OD_{280}/OD_{260})	Report results	1.68

[1]Multiple bands are present on the gel. The lower bands represent the monomeric B-chain and the monomeric A-chain with multiple glycosylation states, as well as smaller peptides that result from the inactivation. The higher molecular weight species represent large insoluble aggregates of the chains that result from the inactivation.
[2]Increased mass due to glycosylation of the A-chain
[3]Performed prior to chemical treatment
[4]Performed with BSA standard curve
[5]Completed with polyclonal antiserum to ricin holotoxin (NR-862)
[6]Determined by the number of cells that survive 48 hours after toxin challenge

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Figure 1 - SDS-PAGE

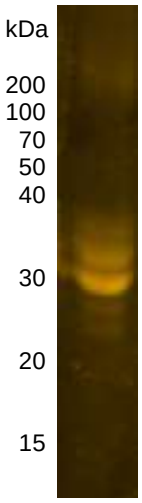
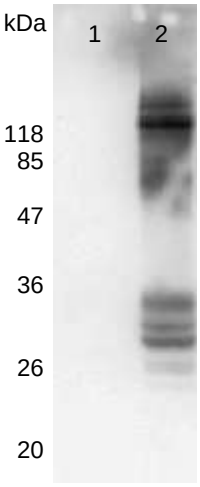
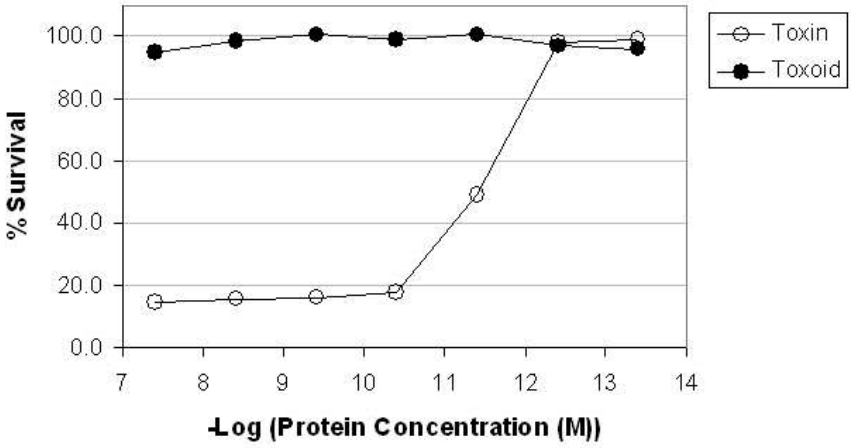


Figure 2 – Western Blot



Lane 1: Carbonic anhydrase
Lane 2: NR-4671

Figure 3 – Vero Cell Cytotoxicity Assay



Date: 21 NOV 2014 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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