

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: NR-4671 was generated by formaldehyde treatment of the purified, glycosylated, ricin holotoxin. Ricin toxoid is non-toxic.

Lot¹: 61667814 Manufacturing Date: 10JUN2014

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 represents 98% of total staining ² (Figure 1)
SELDI-TOF Mass Spectrometry	Measured value within 5% of theoretical value	Measured value (64.9 kDa) within 7% of theoretical value (60.2 kDa) ³
SELDI-TOF Mass Spectrometry of Trypsin Digest	> 50% of total residues accounted for in peptides of expected mass	51% of total residues accounted for in peptides of expected mass
Concentration by BCA Assay ⁴	1.0 mg/mL ± 5%	1.0 mg/mL
Absorbance Ratio (OD ₂₈₀ /OD ₂₆₀)	Report results	1.4
Functional Activity by Western Blot ⁵ Ricin toxoid (NR-4671) Carbonic anhydrase	Reactive Non-reactive	Reactive (Figure 2) ^{2,5} Non-reactive (Figure 2)
Cytotoxicity in Vero Cells ⁶ Ricin toxoid (NR-4671) Ricin (active toxin)	Report results Report results	Non-cytotoxic 2.1 × 10^{-9} M (Figure 3) CD ₅₀ ~ 3 × 10^{-11} M
Sterility	0.22 µm filter-sterilized	0.22 µm filter-sterilized

¹Provided in PBS buffer, 1 mL per vial

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²Multiple 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 formaldehyde inactivation.

³Increased mass due to glycosylation

⁴Performed with Pierce BCA Protein Assay Kit™ and BSA standard curve

⁵Completed with polyclonal rabbit anti-ricin (Sigma cat. no. R-1254)

⁶Determined by the number of cells that survive 48 hours after toxin challenge



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Figure 1: SDS-PAGE

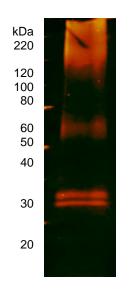
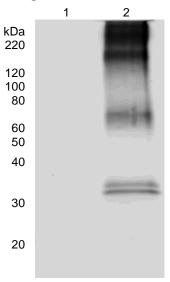
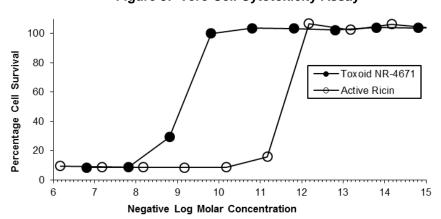


Figure 2: Western Blot



Lane 1: Carbonic anhydrase Lane 2: NR-4671 (1 µg)

Figure 3: Vero Cell Cytotoxicity Assay



Date: 09 OCT 2015

Signature:

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