Certificate of Analysis for NR-2619

Ricin Toxin A Subunit, from *Ricinus communis* Seeds

Catalog No. NR-2619

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

Product Description: Ricin toxin is a glycoprotein that can be isolated from *Ricinus communis* seeds. The A chain of ricin toxin catalytically inactivates the eukaryotic 28S ribosomal RNA subunit resulting in the inhibition of protein synthesis and death of the cell.

Lot[1]: 6176491 **Manufacturing Date: OCT2006**

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless, no particulate matter	Clear and colorless, no particulate matter
SDS-PAGE (SYPRO Orange densitometer scan)	Dominant band of expected size > 95% pure	Dominant band of ~ 30 kDa[2] > 95% pure (see Figure 1)
SELDI-TOF Mass Spectrometry	Report results (expected MW is 30.3 kDa based on amino acid sequence)	Mass is 5.9% greater than expected due to protein glycosylation (32.1 kDa)
SELDI-TOF Mass Spectrometry of Trypsin Digest	> 50% of total residues accounted for in peptides of expected mass	58% of total residues accounted for in peptides of expected mass
Concentration by Bicinchoninic Acid Protein Assay	Report results	0.12 mg/mL
Functional Activity Western Blot (see Figure 2)[3] Carbonic anhydrase NR-2619	 Non-reactive Reactive	 Non-reactive Reactive
Toxicity Cytotoxicity in CCL-81 cells Toxicity in murine model[5]	 Report results Report results	 CD_{50} ~58 nM[4] No lethality in mice
Sterility	0.22 µm filter sterilized	0.22 µm filter sterilized

[1]Purified from NR-720 ricin holotoxin by ion-exchange chromatography.
[2]Actual band may appear larger than predicted from the sequence due to glycosylation; protein doublet is also due to glycosylation.
[3]Completed with mouse monoclonal antibody to ricin A chain (NR-843).
[4]This value is approximately 5 log units less than the CD_{50} reported for the holotoxin.
[5]CD-1 mice were injected with concentrations of NR-2619 that correspond to 10 and 100 LD_{50} of ricin holotoxin (NR-720). No mouse injected with NR-2619 became sick or died, indicating that the ricin toxin subunit A preparation is not lethal to mice.

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Figure 1

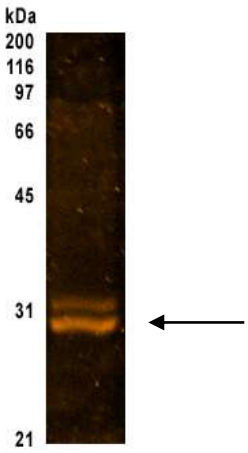
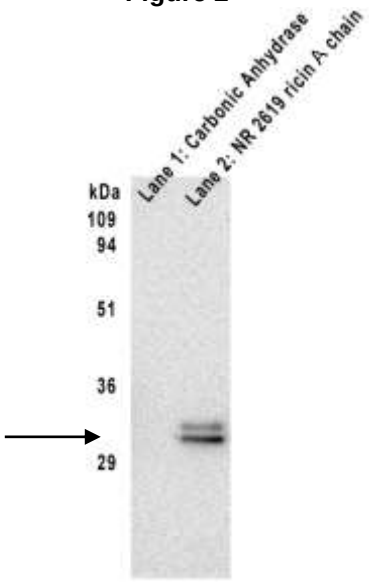


Figure 2



Date: 20 NOV 2014 **Signature:**

Title: Technical Manager, BEI Authentication or designee

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