b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-4367

Anthrax Lethal Factor (LF-HMA), Recombinant from *Bacillus anthracis*

Catalog No. NR-4367

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

Product Description: Recombinant anthrax LF-HMA was produced in a non-sporulating avirulent strain of *Bacillus anthracis* lacking both of the wild type plasmids, pX01 and pX02. Recombinant LF-HMA was purified using conventional chromatographic techniques. The resulting purified protein lacks all other anthrax virulence factors. Note: NR-4367 was vialed using the same bulk LF-HMA as NR-142 and NR-4368. NR-142, NR-4367 and NR-4368 showed similar cytotoxic activity in side-by-side tests.

Lot: 1722B5 **Manufacturing Date[1]: 01AUG2002**

TEST	SPECIFICATIONS	RESULTS
Appearance[2]	Clear and colorless	Clear and colorless
SDS-PAGE (Coomassie Blue Densitometer Scan)	~ 80–90 kDa band is ≥ 90% of total density	~ 80–90 kDa band is 96% of total density
HPLC	Report results (% of protein in full length peak)	14% of protein in full length peak
Electrospray Mass Spectrometry	Report results (expected MW is 90,496 Da)	5 components: 88,000–91,000 Da
Concentration by Modified Bradford Assay[2,3]	Report results	0.73 mg per mL
Functional Activity		
FRET (fluorescence resonance energy transfer) protease assay	Report results (rate of cleavage of MAPKKide™ peptide)	0.321 units/mg
Cytotoxicity assay [determination of effective concentration (EC_{50}) by titration of LF-HMA in J774A.1 macrophage cells]	LF-HMA with 1 µg/mL PA: EC_{50} ≤ 500 pM 1 µg/mL LF-HMA alone: EC_{50} ≥ 10,000 pM 1 µg/mL PA alone: EC_{50} ≥ 10,000 pM	33 pM ≥ 10,000 pM ≥ 10,000 pM
Microbial Content[4]	No detectable colony-forming units in 0.2 mL final product	No detectable colony-forming units in 0.2 mL final product
Endotoxin Content (Limulus Amoebocyte Lysate Assay)	< 0.5 EU endotoxin per µg protein	0.003 EU endotoxin per µg protein
Absorbance Ratio (OD_{280}/OD_{260})	≥ 1.7	2.1
Absorbance Ratio (OD_{280}/OD_{320})	≥ 10	35

[1]Stored as frozen bulk LF-HMA from August, 2002 until November, 2006. Frozen bulk LF-HMA was thawed and aliquoted on 27 November, 2006. Lyophilization was completed on 29 November, 2006.
[2]Prior to lyophilization
[3]Using BSA as a standard
[4]Performed on bulk LF-HMA prior to being frozen in 2002

Date: 05 JUL 2012 **Signature:** Dorothy C. Young

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.

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