

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-570

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

Catalog No. NR-570

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.

Lot: 1724A1A Manufacturing Date: 06AUG2004

TEST	SPECIFICATIONS	RESULTS
Appearance ¹	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)	66% of protein in full length peak
Electrospray Mass Spectrometry	Report results (expected MW is 90,496 Da)	90,493 Da
Concentration by Modified Bradford Assay ^{1,2}	Report results	1.0 mg per mL
Functional Activity FRET (fluorescence resonance energy transfer) protease assay	Report results (rate of cleavage of MAPKKide™ peptide)	0.202 units/mg
Cytotoxicity assay (determination of effective concentration (EC ₅₀) by titration of LF-HMA in J774A.1 macrophage cells)	LF-HMA with 1 μ g/mL PA: EC ₅₀ \leq 500 pM 1 μ g/mL LF-HMA alone: EC ₅₀ \geq 10,000 pM 1 μ g/mL PA alone: EC ₅₀ \geq 10,000 pM	$EC_{50} = 32 \text{ pM}$ $EC_{50} \ge 10,000 \text{ pM}$ $EC_{50} \ge 10,000 \text{ pM}$
Microbial Content	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)	< 0.50 EU endotoxin per μg protein	0.12 EU endotoxin per μg protein
Absorbance Ratio (OD ₂₈₀ /OD ₂₆₀)	≥ 1.7	1.8
Absorbance Ratio (OD ₂₈₀ /OD ₃₂₀)	≥ 10	12

Prior to lyophilization

²Using BSA as a standard

Date: 05 JUL 2012

Signature: Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

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