

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

Catalog No. NR-4368

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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. **NR-4368 was vialled using the same bulk LF-HMA as NR-142 and NR-4367. NR-142, NR-4367 and NR-4368 showed similar cytotoxic activity in side-by-side tests.**

Lot: 1722B6

Manufacturing Date¹: 01AUG2002

TEST	SPECIFICATIONS	RESULTS
Appearance²	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 97% 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	0.75 mg per mL
Functional Activity FRET (fluorescence resonance energy transfer) protease assay Cytotoxicity assay [determination of effective concentration (EC ₅₀) by titration of LF-HMA in J774A.1 macrophage cells]	Report results (rate of cleavage of MAPKKide™ peptide) 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	0.330 units/mg 33 pM ≥ 10,000 pM ≥ 10,000 pM
Microbial Content⁴	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	0.0124 EU endotoxin per µg protein
Absorbance Ratio (OD₂₈₀/OD₂₆₀)	≥ 1.7	2.2
Absorbance Ratio (OD₂₈₀/OD₃₂₀)	≥ 10	62

¹Stored as frozen bulk LF-HMA from August, 2002 until November, 2006. Frozen bulk LF-HMA was thawed, aliquoted and frozen on 27 November, 2006.

²Prior to lyophilization

³Using BSA as a standard

⁴Performed on bulk LF-HMA prior to being frozen in 2002

Date: 05 JUL 2012

Signature:



Title: Technical Manager, BEI Authentication or designee

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