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

Catalog No. NR-723

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For research use only. Not for human use.

Contributor:

BEI Resources

Manufacturer:

List Biological Laboratories, Inc.

Product Description:

Recombinant anthrax lethal factor (LF, 90 kDa) was produced using a plasmid licensed from the NIH.[1,2] The plasmid contains the coding sequences for two additional amino acids, a histidine (H) and a methionine (M), beyond the native N-terminal alanine (A). The lethal factor produced from this plasmid has recently been designated LF-HMA by the manufacturer. The plasmid was introduced into 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.

LF is a zinc-dependent metalloprotease which cleaves the amino terminus of signaling proteins of the mitogen-activated protein kinase family (MAPKK), destroying their ability to signal. *In vivo*, recombinant LF binds to a cleaved form of recombinant protective antigen (PA), and is transported by PA into the cytosol of the macrophage where LF exerts its pathogenic effect.

Material Provided:

Each vial contains approximately 0.1 mg (lyophilized) of recombinant LF-HMA from *Bacillus anthracis.* After reconstitution with 0.1 mL of sterile water, the buffer concentration is 5 mM HEPES (pH 7.5) and 50 mM NaCl. Note: Handle the product gently; DO NOT VORTEX.

Packaging and Storage:

This product was packaged aseptically, lyophilized, and sealed under vacuum. The product is provided at room temperature and should be stored at 2°C to 8°C prior to reconstitution.

Reconstitution and Storage:

Recombinant anthrax LF-HMA in 5 mM HEPES (pH 7.5) and 50 mM NaCl is stable for a few hours at 2°C to 8°C. Longer periods of time at 2°C to 8°C will result in a decline in the activity of PA-LF complex in living cells.

To enhance stability and recovery, reconstitution at 1 mg/mL[1] in the presence of 1 mg/mL bovine serum albumin (BSA) is recommended. Under these conditions, storage for a period of two weeks at 2°C to 8°C may be acceptable for some applications.

For optimal long-term storage, aliquoting and freezing the material at -20°C or colder is recommended. Repeated freeze-thaw cycles should be avoided. Glycerol may be added to 50% if a liquid is desired at freezer temperatures.

Tissue Culture Application:

Tissue culture media containing glutamate must be fresh. Ammonium ion released when glutamate breaks down may prevent acidification of the endosome thereby inhibiting translocation of LF or edema factor (EF) into the cytosol.[2] A stable form of glutamate may be used.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Anthrax Lethal Factor (LF-HMA), Recombinant from *Bacillus anthracis*, NR-723."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Leppla, S. H. "Production and Purification of Anthrax Toxin." Methods Enzymol. 165 (1988): 103-116. PubMed: 3148094.
2. Stephen Little, personal communication.

Support Provided by NIAID

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