Anthrax Lethal Factor (LF-A), Native Sequence, Recombinant from *Bacillus anthracis*

Catalog No. NR-28544

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

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) containing the native alanine (A) at the N-terminus was produced using a plasmid licensed from the NIH.[1-3] The lethal factor produced from this plasmid has been designated LF-A 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-A was purified using conventional chromatographic techniques. The resulting purified protein lacks all other anthrax virulence factors.

A recombinant form of the LF protein containing two additional amino acids at the N-terminus, a histidine (H) and a methionine (M), beyond the native N-terminal alanine (A), has been or is currently available from BEI Resources (NR-142, NR-404, NR-570, NR-723, NR-724, NR-2673, NR-4367, NR-4368). This form of the protein has been recently designated LF-HMA by the manufacturer and is also produced in *Bacillus anthracis*. Note: LF-A has been reported to have 3-fold higher potency than LF-HMA in cell culture cytotoxicity assays and in rat lethality studies.[3]

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-A 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-A 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-A), Native Sequence, Recombinant from *Bacillus anthracis*, NR-28544."

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/BMBL.pdf.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S.

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Leppla, S. H. "Production and Purification of Anthrax Toxin." Methods Enzymol. 165 (1988): 103-116. PubMed: 3148094.
2. Stephen Little, personal communication.
3. Gupta, P. K., et al. "Role of N-Terminal Amino Acids in the Potency of Anthrax Lethal Factor." PLoS One 3 (2008): e3130. PubMed: 18769623.

ATCC® is a trademark of the American Type Culture Collection.

Support Provided by NIAID

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898