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ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Lethal Factor (LF) Protein

Cat. # LF15-R Recombinant purified LF Protein **SIZE**: 100 ug

After inhalation by mammals, *Bacillus anthracis* spores germinate in alveolar macrophages then migrate to lymph nodes where they multiply. The vegetative bacteria excrete the tripartite exotoxin which consists of three polypeptides: protective antigen (**PA**, 83 kDa), lethal factor (LF, 90 kDa) and oedma factor (OF, 89 kDa). The two components (OF and LF) of the toxin enzymatically modify substrates within the cytosol of the mammalian cells: The **OF** is an adenylate cyclase that impairs the host defenses through a variety of mechanisms inhibiting phagocytosis. The **LF** is a zinc dependent protease that cleaves several mitogen activated protein kinase kinases **(MAPKK)** and causes lysis of macrophages. To intoxicate mammalian cells, the third component of the toxin **PA,** binds to a ubiquitously expressed cellular receptor, Tumor Endothelium Marker-8 (**TEM8**). Upon binding to TEM8, PA is cleaved into 20 and 63kDa fragments (PA20 and PA63) by furin or furin-like proteases. PA20 dissociates into medium and allows the PA63 fragment to heptamerize and bind LF and OF of the toxin. The resulting complex of **PA63** fragment with EF and/or OF binds to PA-receptor TEM8/ATR and internalized into endosomes followed by translocation of LF and OF into cytosol of the cells.

Anthrax lethal toxin produced by the bacterium *Bacillus anthracis* is the major cause of death in animals infected with **anthrax**. One component of this toxin, **lethal factor** (LF), inactivates members of the mitogen-activated protein kinase kinase or MEK family through proteolysis of their NH_2 termini. Although LF has been shown to cleave the NH_2 termini of select members of the mitogen-activated protein kinase kinase or MEK family, the substrate requirements that determine LF specificity are unknown. Indirect evidence suggests that epitopes distal to the cleavage site are required for LF-MEK interaction.

B. anthracis Lethal Factor (LF) is comprised of four domains: domain I binds the protective antigen to enter the target cell, domain II, III and IV create a long groove to hold and cleave the MAPKK proteins heptamer to insert into the membrane and form a water filled channel.

Source of Protein

LF is supplied as highly purified, single major band by SDS-PAGE, Mol wt 90000. It is provided as 100ug lyophilized solid. The lyophilized products should be reconstituted with 1.0 ml of sterile distilled water. However, the preparation is not necessarily sterile. The solution can be sterile filtered, if necessary. It can then be used or aliquoted for storage in small aliquots at -70oC or below. Lyophilized product can be stored at 4oC or –20oC for up to 1-2 years. After reconstitution, store at –20oC for up to 6-months.

Biological activity and Cytotoxicity

Activity of LF is determined by using a synthetic peptide containing a single cleavage site for LF. One unit is defined as the amount of lethal factor needed to catalyze the release of 1.0 umole of cleaved MAPKKide™ per minute at 37℃ in 20 mM HEPES, pH 8.2. The specific activity of a typical lot of lethal factor is 0.3-0.4 units/mg.

LF is also tested for cytotoxicity in the presence of 1 ug/ml PA using J774A1 cells. LF alone is not toxic. The effective concentration 50% (EC_{50}) of this LF lot meets specifications.

Suggested uses:

It can be used for ELISA or Western for positive control. Monoclonal antibodies to LF (cat # LF11-M and LF12-M) are also available.

General References Arun P. Chopra et al (2003) JBC Vol. 278, Issue 11, 9402-9406; Sung O et al (2003) JBC Vol. 278, 7413-7421; Bradley KA et al (2001) Nature 414, 225-229; liu S and Leppla SH (2002) JBC (in press); Leppla, SH (1982) PNAS 79, 3182; O'Brien J et al (1985) Infect Immun 47, 306; Duesbery, NS et al (1998) Science 280, 734.

MSDS Information

Anthrax proteins (LF, PA, and EF are produced using non-sporulating avirulent strain of Bacillus anthracis which lacks both of the wild type plasmids, pX01 and pX02. This host makes none of the anthrax toxin components and no polygutamate capsule. The purified proteins are homogenous void of all other virulence factors. Purified proteins are non-toxic and presents no special hazard during normal laboratory use. However, all material should be used by trained personnel and use GLP procedures when handling and disposing the products.

**This product is for In vitro research use only.*

Related materials available from ADI

Antibodies: ATR11-A, ATR12-A, ATR31-A, Protective antigen A, Spore Antigen Edema factor etc.

Recombinant purified PA63, PA83, Lethal factors, Edema factor; Poly and mono antibodies are also available.

LF15-R 90805A

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