ANTHRAX TOXIN
PROTECTIVE ANTIGEN, LETHAL FACTOR, EDEMA FACTOR
From *Bacillus anthracis*

Anthrax toxin is responsible for the symptoms associated with anthrax disease.[1] Inhaled *B. anthracis* spores lodge in the lungs where they are ingested by macrophages. Within macrophages, spores germinate, multiply and eventually kill the cell. Infected macrophages migrate to the lymph nodes where, upon death, they release their contents, allowing vegetative *B. anthracis* to enter the bloodstream, multiply rapidly and secrete a lethal dose of toxins.[2]

Three proteins are collectively known as anthrax toxin: protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa) and edema factor (EF, 89 kDa). These proteins play a key role in the pathogenesis of anthrax. EF and LF have enzymatic functions associated with their carboxyl terminals, but require PA to achieve their biological effects.[3] The amino terminal ends of EF and LF bind to PA and are responsible for translocation through the pore formed by the PA heptamer. The receptor for PA is present on many cell types and has been identified.[4,5] Combined PA and LF, known as lethal toxin (LeTx), causes death when injected intravenously in animals. Lethal toxin is active in a susceptible cell culture line of macrophages causing cell lysis within a few hours.[6,7] It can induce both necrosis and apoptosis in mouse macrophage-like J774A.1 cells.[8] Edema factor associates with PA to produce edema toxin (EdTx), which when injected intradermally causes edema in the skin.[9] CHO cells are sensitive to edema toxin.

Protective antigen, the receptor binding component of anthrax toxin, is responsible for transporting the other two factors into the host cell. PA 83 monomer, an 83 kDa protein, is secreted from rapidly growing *B. anthracis* cells where it is cleaved by cellular furin-like proteases releasing a fragment (PA 20) from the N-terminal.[6] The remaining portion, PA 63, may oligomerize into a ring-shaped heptamer.[10] Although the C-terminal regions of both PA 63 and PA 83 are capable of binding to the cell receptor, cleavage of PA is an essential step in exposing the binding sites for EF and LF. Cleavage also allows the formation of the heptamer. Each heptamer attached to the surface of a cell has the ability to bind up to three molecules of LF and/or EF. The complex formed between PA heptamer and EF or LF is taken into the cell by receptor mediated endocytosis.[11] Following endocytosis, the acidified environment within the endosome triggers the heptamer to act as a pore, releasing LF and EF into the cytosol where they attack their targets. It appears that EF may remain associated with the vesicle membrane after translocation.[12]

Lethal factor is a zinc dependent metalloprotease which cleaves a specific bond in signaling proteins of the mitogen-activated protein kinase kinase family (MAPKK), destroying their ability to signal.[13,14] LF cleaves the amino terminus of MAPKKs. Of the seven different MAPKKs, six are cleaved by LF.[15,16] The crystal structure of LF complexed with the N-terminal portion of MAPKK-2 has been described.[17] Within the cell, MAP kinase pathways are involved in the transduction of a variety of signals including those involved in cell proliferation and differentiation.[18] Edema factor is a calmodulin-dependent adenylate cyclase.[19] After cell entry, EF is activated when complexed with calmodulin, creating excess amounts of cellular cyclic-AMP. The increase in cyclic-AMP upsets water homeostasis and disrupts the intracellular signaling pathways. It is responsible for the swelling in cutaneous anthrax infections.

Refer to Ordering Information below for a list of anthrax products. All are produced recombinantly in the native host. Both PA (prod. **#171**) and LF (LF-A, prod. **#169**) contain the expected sequences. Mutant LF E687C (prod. **#176**) has a Glu to Cys substitution in the zinc-binding site,[21,22] eliminating enzymatic activity.[13,16] Anthrax edema factor (prod. **#178**) is comprised of the native sequence with an additional amino acid, histidine, on the N-terminal. Mutant EF S414N (prod. **#173**) has reduced adenylate cyclase activity and has been shown to act as an adjuvant.[25] Protective antigen is also trypsin activated and purified, producing the active binding PA 63 (prod. **#174**). PA 20 (prod. **#177**), the 17-20 kDa cleavage fragment resulting from trypsin activation of PA, is also available.

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Other products available include PA 63 conjugated to fluorescein isothiocyanate, FITC (prod. **#175**). Antibodies available include goat anti-PA, LF, and EF suitable for binding and detection assays. Additionally, an affinity purified goat anti-PA antibody is available. We also offer a chicken IgY antibody raised against Product #169, LF-A, with or without biotin. FRET peptide substrates containing a cleavage site for LF are available as MAPKKide®, products **#530**, **#531**, **#532**. Unquenched calibration peptide (prod. **#539**) is offered to be used with the o-Abz/Dnp FRET peptide (prod. **#530**). MAPKKide® Plus* (prod. **#532**) is a fluorescently labeled peptide substrate specific for LF and resistant to cleavage by nonspecific proteases found in plasma. This substrate is highly sensitive to LF and may be used to detect early anthrax infections in plasma. PA and LF are also offered as Quality Documented (QD) products which are manufactured and tested using well controlled and documented procedures compliant with Q7A methodology. All QD lots pass multi-test quality control and the results accompany each shipment.

Recombinant PA, EF and LF are prepared by List Biological Laboratories, Inc. using a modification of the production and purification methods of Leppla and Park *et al.*[23,24] Each protein is produced separately in an avirulent, non-capsulated, sporulation-suppressed or sporulation deficient *B. anthracis* host. Purity is checked by gel electrophoresis. These products are supplied as lyophilized powder or frozen liquid formulations, and a detailed lot analysis with instructions on reconstitution, storage and handling is provided with each shipment.

* Patent Pending

These products are intended for research purposes only and are not for use in humans or as diagnostic agents. For further information, please contact List Biological Laboratories, Inc.

Ordering Information

Product No.	Description	Sizes
	Protective Antigen	
171 D,E	Anthrax Protective Antigen (PA), Recombinant from *Bacillus anthracis* All protective antigen products are produced recombinantly in the native host.	50 µg, 0.5 mg
174	Anthrax Protective Antigen, Activated (PA 63) from *Bacillus anthracis*	0.5 mg
175	Anthrax PA 63 – Fluorescein Isothiocyanate Conjugate (FITC)	50 µg
177 A,B	Anthrax Protective Antigen Fragment (PA 20)	50 µg, 0.25 mg
9171 B	QD Grade Anthrax Protective Antigen (PA), Recombinant from *Bacillus anthracis.* Manufactured and tested compliant with Q7A methodology. Multi-test quality control results provided with each purchase. Produced recombinantly in the native host.	1 mg
	Lethal Factor	
169 A,B	Anthrax Lethal Factor (LF-A) Native Sequence from *Bacillus anthracis* Produced recombinantly in the native host, with the native alanine N-terminal. This is the preferred form for most research.	0.1 mg, 1 mg
9169 A,B	QD Grade Anthrax Lethal Factor (LF-A) Native Sequence from *Bacillus anthracis*	0.1 mg, 1 mg
172 B,C,D	Anthrax Lethal Factor (LF), Recombinant from *Bacillus anthracis* Produced recombinantly in the native host with two additional amino acids on the N-terminal.[20] Some lots of this product were cleaved during manufacturing producing a mixture of LF with native and altered N-terminals.	1 mg, 10 mg, 70 µg
176 A,B	Lethal Factor (LF), Recombinant Mutant E687C from *Bacillus anthracis* Produced recombinantly in the native host. This mutant has a Glu to Cys substitution located at position 687 in the zinc binding site, eliminating enzymatic activity.[13,16,21,22]	0.1 mg, 1 mg

9172 A,B	QD Grade Anthrax Lethal Factor (LF), Recombinant from *Bacillus anthracis.* Manufactured and tested compliant with Q7A methodology. Multi-test quality control results provided with each purchase. Produced recombinantly in the native host.	0.1 mg, 1 mg
	Edema Factor	
178 A	Anthrax Edema Factor (EF), Recombinant from *Bacillus anthracis* Edema factor is produced recombinantly in the native host.	0.1 mg
173B	Anthrax Edema Factor (EF), Recombinant Mutant S447N from *Bacillus anthracis.* Produced recombinantly in the native host, this mutant has a Ser to Asn substitution located at position 447 decreasing adenylate cyclase activity.[25]	0.5 mg
	LF Substrates	
530	MAPKKide® Peptide Substrate (o-Abz/Dnp) for *Bacillus anthracis* LF	200 nmoles
531	MAPKKide® Peptide Substrate (DABCYL/FITC) for *Bacillus anthracis* LF	200 nmoles
532	MAPKKide® Plus* (AMC) Specific Substrate for Anthrax Lethal Factor	100 nmoles
539	MAPKKide® Unquenched Calibration Peptide for #530 substrate for *Bacillus anthracis* LF	50 nmoles
	Antibodies for Anthrax Toxin Components	
768L	Anti-Lethal Factor from *Bacillus anthracis* (Chicken lgY), Biotinylated, Liquid	0.1 mg
769 A,B	Anti-Lethal Factor from *Bacillus anthracis* (Chicken lgY)	0.1 mg, 0.2 mg
771 B	Goat Anti-Protective Antigen from *Bacillus anthracis*	1 mg
781	Goat Anti-Protective Antigen from *Bacillus anthracis,* affinity purified	0.1 mg
772 B	Goat Anti-Lethal Factor from *Bacillus anthracis*	1 mg
773 L	Goat Anti-Edema Factor from *Bacillus anthracis,* Liquid	1 mg

* Patent Pending

See how others have used List Labs' products on our citations page: https://www.listlabs.com/citations

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