list labs
www.ListLabs.com

Anthrax Toxins

1. List Labs' Anthrax Proteins are:

Three proteins are collectively known as anthrax toxins: protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa) and edema factor (EF, 89 kDa). Combined PA and LF, are known as lethal toxin (LeTx). Edema factor associates with PA to produce edema toxin (EdTx).

LIS171 - Anthrax Protective Antigen (PA)
LIS169 - Anthrax Lethal Factor (LF-A)
LIS178 - Anthrax Edema factor (EF)

2. Key attributes

Toxins are recombinant, produced in a modified *Bacillus anthracis* host and provided at >90% purity. Assay results for cytotoxicity and enzyme activity are provided for each lot of EF and LF. Results for PA describe its ability to deliver LF and cause cytotoxicity. Spores are the infectious form of *B. anthracis*, and they are not present in the purified toxins.

3. Additional products supporting anthrax research

Protective antigen: In addition to the intact PA (171), List Labs offers the two parts of protective antigen formed during activation:

LIS153 - Anthrax Protective Antigen Fragment, PA20
LIS154 - Anthrax Protective Antigen Activated, PA63
LIS175 - Anthrax Protective Antigen Activated, PA63, FITC Conjugate

Lethal Factor, other forms:

LIS172 - Anthrax Lethal Factor (LF-HMA) is List's first LF product, but it was found to carry two additional amino acids on the N-terminal, an artifact of genetic engineering. LIS169 listed above has the native N-terminal and should be chosen by new users.
LIS176 - Anthrax Lethal Factor, Mutant E687C, has been deliberately mutated to remove the enzymatic function.

Antibodies: several antibodies are provided for work with the toxins

LIS768, LIS769, LIS772 - Anti-LF made in chicken and goat, one is biotinylated
LIS781 - Anti-PA made in goat and affinity purified
LIS773 - Anti-EF from goat

Substrates: peptides modeled after the natural substrate for LF are used to detect the LF enzyme. These peptides, trademarked MAPKKide®, fluoresce when cut by LF.

LIS530 - MAPKKide® peptide substrate for LF tagged with o-Abz/Dnp
LIS539 - Unquenched calibration peptide to use with #530
LIS531 - MAPKKide® peptide substrate for LF tagged with DABCYL/FITC
LIS532 - MAPKKide® Plus Specific Substrate tagged with AMC

4. Specific requirements

Anthrax toxins can be handled in a laboratory setting using good laboratory techniques.

5. Technical information

Briefly, anthrax toxins are interesting research tools because, they…

- Are the primary virulence factors of *B. anthracis*. PA and LF form lethal toxin, and PA and EF form edema toxin. This three-part toxin is a major virulence factor and thus a prime therapeutic target for treatment of anthrax.
- Modify MAP kinase kinases, which are important in directing cellular responses and can be used to study these processes.
- Represent the family of bacterial toxins and function using many processes of intoxication: selective binding, endocytosis, pore formation, translocation, and modification of the host machinery.

More information about anthrax toxins:

- Anthrax is deadly disease, so some people are reluctant to work with the toxins. These toxins need to be handled using good laboratory practices but are relatively safe. The infectious form of anthrax is the spore, and our products are free of spores. Anthrax toxins are useful for researchers working to detect the toxin or to test therapies for treatment.
- On an infected cell, protective antigen is cleaved and shortened to form PA63 and seven or eight of these molecules cluster together to form a pore which allows the transfer of either LF or EF into the cell.
- In the cytoplasm of the cell LF, a metalloprotease, cleaves members of the MAP kinase kinase family (MEK, MAPKK & MAP2K), causing their inactivation. This sets off a chain reaction, disabling regulatory pathways such as ERK, JNK and p38 pathways. This property of anthrax LF makes it a useful tool for cell biologists.
- EF is an adenylyl cyclase, an enzyme capable of converting up to 2000 molecules of ATP to cAMP per second, significantly increasing the concentration of this second messenger and causing edema. Like LF, EF can be used as a tool to create changes within cells and lead to discoveries about cell function.

Recent research with anthrax toxins:

Anthrax toxin can enter living cells, and the toxin enzymes, LF and EF make known changes. Because of this activity, anthrax toxins are valuable tools to investigate cell processes. Some of the work already accomplished with these toxins can be found in the following selected references:

- Work with anthrax toxins is providing a better understanding of cellular processes. PA binds specifically to two toxin receptors, tumor endothelial marker 8 (TEM8, also called ANTXR1) and capillary morphogenesis 2 (CMG2, or ANTXR2). Several other factors are involved in the internalization of anthrax toxin.

  Abrami L, Bischofberger M, Kunz B, Groux R, van der Goot FG (2010) Endocytosis of the Anthrax Toxin Is Mediated by Clathrin, Actin and Unconventional Adaptors. PLoS Pathog 6(3): e1000792. **PMID: 20221438**

- Anthrax vaccines are currently under development and demonstration that antibodies that will neutralize anthrax toxin is essential.

  Laws TR, Kuchuloria T, Chitadze N, *et al* (2016) A Comparison of the Adaptive Immune Response between Recovered Anthrax Patients and Individuals Receiving Three Different Anthrax Vaccines. PLoS One 11(3):e0148713. Published 2016 Mar 23. doi:10.1371/journal.pone.0148713 **PMID: 27007118**

- PA could potentially deliver polypeptides and compounds to the cell cytoplasm. In the two studies described in the following papers, PA was used as a tool to deliver biochemicals to the cytoplasm of eukaryotic cells.

  Dyer PDR, Shepherd TR, Gollings AS, *et al* (2015) Disarmed anthrax toxin delivers antisense oligonucleotides and siRNA with high efficiency and low toxicity. J. Control. Release 2015, 220, 316–328. **PMID: 26546271**

  Rabideau, AE, Liao XL, Akcay G, Pentelute BL (2015) Translocation of Non-Canonical Polypeptides into Cells Using Protective Antigen. Sci Rep 5: 11944, **PMID:26178180**, **PMCID: PMC 4503955**

- PA has been used to target cancer cells overexpressing TEM8. This receptor has been shown to be upregulated during tumor angiogenesis and provides a convenient target for anti-angiogenic therapy.

  Chen KH, Liu S, Bankston LA, Liddington RC, Leppla SH (2007) Selection of anthrax toxin protective antigen variants that discriminate between the cellular receptors TEM8 and CMG2 and achieve targeting of tumor cells. J Biol Chem 282: 9834–9845. **PMID: 17251181**

  Chaudhary A, Hilton MB, Seaman S, *et al* (2012) TEM8/ANTXR1 blockade inhibits pathological angiogenesis and potentiates tumoricidal responses against multiple cancer types. Cancer Cell 21:212–226. **PMID: 22340594**