LIST BIOLOGICAL LABORATORIES, INC.

540 DIVISION STREET • CAMPBELL • CALIFORNIA 95008-6906 • USA
408-866-6363 • 800-726-3213 • FAX 408-866-6364 • EMAIL info@listlabs.com
WEBSITE www.listlabs.com

Lethal Factor from *Bacillus anthracis,* Native Sequence

List Biological Laboratories has been at the forefront, producing reagents for anthrax research since 2002. Our newest product, #169 **Lethal Factor (LF) from *Bacillus anthracis,* Native Sequence,** is produced recombinantly in the native host rather than in a non-native host such as *E. coli* or *Bacillus subtilis.* Product #169 replaces our previous offering, product #172, Anthrax Lethal Factor (LF), Recombinant from *Bacillus anthracis.*

LF when combined with Protective Antigen (PA) forms Lethal Toxin, a major virulence factor for *Bacillus anthracis.* PA is the receptor-binding component for Lethal Toxin, which delivers LF to the cytosol where LF is enzymatically active. Lethal Toxin induces lethal vascular collapse in animals similar to that observed in anthrax infections.

The new construct is described in Gupta, PK, Moayeri, M. Crown, D, Fettah, RJ, Leppla, SH, "Role of N-Terminal Amino Acids in the Potency of Anthrax Lethal Factor," Plos ONE, 3(9):e3130, 2008. Our new product #169 is designated LF-A in this citation indicating the native alanine N-terminal, and product #172 is designated LF-HMA for the N-terminal with two additional amino acids beyond the alanine, histidine and methionine. LF-HMA has three-fold lower potency than LF-A in cell culture cytotoxicity assays and in rat lethality studies. This observation is explained by the "N-end rule" effect wherein the half-life of proteins in cells correlates with the identity of the protein's N-terminal amino acid. Thus, the two artifactual amino acids added to LF-HMA by cloning manipulations, decreased cytosolic stability of LF-HMA and resulted in decreased potency.

Gupta *et al* showed that the difference in activity for LF-HMA and LF-A is not due to altered binding to PA; since both had similar affinities in binding studies and there was no evidence that translocation was different. *In Vitro* enzymatic activity measured using the synthetic FRET substrate MAPKKide® (List product #530) is expected to be equivalent for product #169 and #172. LF is a zinc-dependent metalloprotease that cleaves several MAPK kinases.

Various expression hosts have been used to produce LF by others, including *E. coli, Bacillus subtilis* and *B. anthracis.* Different potencies have been seen with LF from various sources. Gupta, *et al,* recommend production of LF in the native host. Secretion to the culture supernatant from the native host organism assures that processing by the signal peptidase and folding is both optimal and native. List's LF is identical to the native sequence for LF and has equivalent cytotoxic and lethal activity as the *B. anthracis* Sterne strain. Production of native sequence LF recombinantly in the native host *B. anthracis,* cured of virulence plasmids pXO1 and pXO2, assures the highest quality LF without the concern of contamination by the other toxin components, PA or Edema Factor (EF). List is committed to consistently generating the highest quality LF with native sequence and activity, so important to basic research and development of vaccines and therapeutics.