ALPHA TOXIN FROM *CLOSTRIDIUM SEPTICUM*

Alpha toxin from *Clostridium septicum* is a member of the family of β-pore forming toxins (β-PFTs) and is identified as the main virulence factor of this bacterium, well known for causing non-traumatic gas gangrene in humans and animals.[1-3] The toxin is produced as a single, polypeptide, secreted protein. Seven of the toxin monomers oligomerize on mammalian cell membranes creating a ring-structured trans-membrane pore/channel.[2] The pore allows the efflux of K^+ and influx of Ca^+ which lead to osmotic cell lysis and death.[3] This hydrophilic pore produced by *C. septicum* alpha toxin is estimated to be 1.3-1.6 nm in diameter.[4] The β-PFT family includes β and ε-toxins of *C. perfringens*,[5,6] aerolysin[7] and alpha toxin of *Staphylococcus aureus*.[8]

Although *C. septicum* is a part of the gut flora of humans and other animals, infection is often found in patients having pre-disposing medical conditions such as colonic carcinoma, defects of the bowel, leukemia, diabetes, peripheral vascular diseases, skin infections/burns and septic abortions. Once infected, toxin mediated myonecrosis progresses very rapidly with extensive tissue damage, edema, thrombosis and fluid-filled bullae with a mortality rate of ~ 80% in adults, typically occurring within 48 hrs of infection.[9]

C. septicum alpha toxin produced at List Labs is a recombinant protein expressed in *E. coli* and is purified as a pro-toxin of about 50 kDa protein. The pro-toxin has been activated by trypsin that nicks before the 45 amino acid residue from the C-terminus ($KRRGKR_{398}SVD$).[10] On SDS-PAGE under reducing conditions, active toxin appears to be about 45 kDa protein. A wide variety of nucleated mammalian cells, including IEC-6 (EC_{50} 5.8 ng/ml), Vero (EC_{50} 24.3 ng/ml), CHO (EC_{50} 24.3 ng/ml), MDCK (EC_{50} 14.6 ng/ml), and MDBK (EC_{50} 24.3 ng/ml), have been reported to be sensitive to this toxin.[11] The toxin appears to target and utilize detergent resistant membranes (DRMs) for binding and subsequent oligomerization.[11] Tachyzoites of *Toxoplasma gondii,* a parasitic protozoan that infects a broad range of warm-blooded hosts including humans, was found to be very sensitive to alpha toxin (EC_{50} 0.2 nM).[12] Plasma membrane ruffling and blebbing were observed in toxin treated tachyzoites cells, followed by a microtubule dependent fragmentation and vacuolation of endoplasmic reticulum.[12]

C. septicum alpha toxin is a valuable reagent and research tool to use in various settings:

Cancer research: Alpha toxin has recently been used in cancer research to screen and identify a number of tumor antigens.[13,14] The toxin binds to certain glycosyl phosphatidyl inositol (GPI) anchored proteins on the mammalian cell surface which are identified as receptors for this toxin.[15] Several of these GPI-anchored proteins are also identified as tumor antigens, such as carcinoembryonic antigen, mesothelin, prostate-specific stem cell antigen, and urokinase plasminogen activator receptor. These antigens have also been reported to be elevated in blood plasma of breast, ovarian, kidney, liver and brain cancer patients. Alpha toxin has been used to capture these reporter antigens, and a number of them have been identified by mass spectrometric analysis.[13,14] The toxin can be used as a tool to screen and detect these tumor antigens and find new biomarkers/targets for various cancers.

GPI-anchored protein synthesis and trafficking: Alpha toxin binds to GPI-anchored proteins, including surface antigen 1 (SAG1) and SAG3 of several protozoan parasites.[12] The toxin has been used to understand how GPI-anchored proteins are synthesized and transported through the unusual triple-membrane structure of the parasite pellicle to the plasma membrane.[12] Alpha toxin can be used as an experimental tool for molecular genetic analysis of GPI-anchor biosynthesis and GPI-anchored protein trafficking in *T. gondii* and other susceptible protozoa.[12]

Development of immunoassays, vaccines and understanding the pathophysiology of the disease: Farm animals and birds (commercial turkeys) are very susceptible to *C. septicum* infection if they are not vaccinated or treated immediately after the onset of infection. Infection often occurs from deep puncture wounds, castration and navel infections in newborn calves.[16,17] A current study reports evidence for *C. septicum* as a primary cause of cellulitis, an acute diffuse infection of the dermis and subcutaneous tissue with edema, in commercial turkeys, and is associated with substantial economic loss to turkey producers.[16,17] Alpha toxin can be used to develop

immunoassays. Alpha toxin has been reported to be an immune dominant extracellular antigen.[18] Inactive toxin/toxoid, therefore, can be used to develop effective vaccines against *C. septicum* mediated diseases. Purified alpha toxin can also be used to understand the disease progression and in pharmacokinetic studies in animal models.

C. septicum alpha toxin from List Labs is in liquid form with 20% glycerol and is highly pure. **This product is intended for research purposes only and is not for use in humans or as a diagnostic agent. For further information, please contact List Biological Laboratories, Inc.**

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Product No.	Description	Size
116L	Alpha toxin from *C. septicum*, recombinant	50 µg

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