PRODUCT INFORMATION

ADENYLATE CYCLASE TOXIN
from *Bordetella pertussis*

Adenylate cyclase (AC) toxin is an important virulence factor of *Bordetella pertussis,* the organism which causes whooping cough, and is a novel research tool for manipulation of cAMP levels in mammalian cells.[1] AC toxin targets myeloid phagocytes, such as neutrophils, macrophages or dendritic cells, bearing the $\alpha_M\beta_2$ integrin CD11B/CD18.[21] AC toxin is a single polypeptide, AB-type bacterial toxin which has the ability to interact with target cells, insert into the cytoplasmic membrane and deliver its adenylate cyclase enzymatic domain to the cell interior.[2,3] The cell entry process, unlike that of many other toxins, does not involve receptor-mediated endocytosis. At the cell surface, AC toxin molecules oligomerize into cation selective pores which mediate calcium influx and potassium efflux.[21] AC toxin is a calcium-binding protein and its ability to intoxicate target cells is calcium-dependent. Once entry has occurred, the enzymatic activity of the toxin (production of cAMP from host cell ATP) is stimulated by endogenous calmodulin.[4] The resultant increase in intracellular cAMP is a function of the concentration of toxin employed, but can easily reach supraphysiological levels and modify cell activities accordingly. This subverts cellular signaling networks and causes phagocyte impotence. AC-mediated Ca^{2+} influx further induces calpain-mediated cleavage of talin that tethers CD11b/CD18 to actin. This enables AC piggybacking on the β2-integrin into lipid rafts of target membrane, where translocation of AC domain across the cell membrane is completed. The elevated cAMP concentrations produced in neutrophils and macrophages by AC toxin causes a profound block in their ability to respond with an oxidative burst when exposed to a particulate or soluble stimulus and reduces complement mediated opsonophagocytosis.

Higher concentrations of AC toxin cause apoptosis, cell cycle arrest at G_1-S and cytotoxicity.[5,21] The effects can be seen visually as PMN membrane ruffling. In dendrocytes, AC toxin induces partial maturation that affects the capacity of dendrocytes to present antigens to T lymphocytes and stimulate adaptive cellular immune response.[21] At the same time AC toxin up-regulates production of a confounding blend of pro- and anti-inflammatory cytokines and chemokines. Relocation into membrane rafts further allows AC toxin to escape rapid endocytic removal from cell surface, thus enabling a subpopulation of AC toxin molecules to oligomerize into cation-selective pores and permeabilize cells for potassium efflux. This yields NALP3 inflammasome activation and leads to IL-1β secretion. In addition to causing intoxication via cAMP accumulation, AC toxin is also hemolytic for ovine and other types of erythrocytes, apparently by producing a pore in the target cell membrane.[6-8]

The toxin gene, cyaA, has been cloned and sequenced by Glaser et al.[9] It shares significant homology in the carboxyl terminal glycine-rich repeat, calcium-binding region with other members of the RTX (repeats in toxin) family hemolysins and leukotoxins from gram negative bacteria.[10,11] The molecular weight of the AC toxin is 177 kDa, as calculated from its amino acid sequence and determined by sedimentation,[9,12] but on SDS-PAGE, it migrates with an apparent molecular weight of 200-216 kDa.[13-15] The toxin is inactive without a post-translational modification involving acylation of one or more sites by the product of an accessory gene, cyaC.[16,17] In the absence of cyaC, the toxin has full enzymatic activity, but is unable to enter and intoxicate cells or to lyse erythrocytes.[18]

Adenylate cyclase toxin from List Biological Laboratories, Inc. is isolated and purified from *E.coli*.[15] Typical purity is approximately 90%, as assessed by polyacrylamide gel electrophoresis run in the presence of SDS. Each lot is tested for both enzymatic activity and cytotoxicity. Adenylate cyclase toxin is provided as a lyophilized powder, sealed under vacuum. When reconstituted with 50 µl of sterile distilled water, each vial contains 50 µg of adenylate cyclase toxin in 20 mM HEPES, pH 7.5, and 8 M urea. A detailed lot analysis documenting purity and biological activity plus complete instructions on reconstitution and storage accompany each shipment.

LIST BIOLOGICAL LABORATORIES, INC.
(408) 866-6363 • (800) 726-3213 • FAX (408) 866-6364
WEBSITE: www.listlabs.com • EMAIL: info@listlabs.com

Edema factor (EF) from *Bacillus anthracis* also has adenylate cyclase activity. It is delivered into cells using protective antigen (PA). Refer to the anthrax toxin product literature for information about EF, product #173.

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

Ordering Information

Product No.	Description	Size
188	Adenylate Cyclase Toxin from *Bordetella pertussis*	50.0 µg
173B	Edema Factor (EF), Recombinant, from *Bacillus anthracis*	0.5 mg

References

1. Weiss, A.A. and Hewlett, E.L. (1986) *Annu. Rev. Microbiol.* **40**, 661-686.

2. Mock, M. and Ullmann, A. (1993) *Trends in Microbiol.* **1**, 187-192.

3. Hewlett, E.L., and Maloney, N.J. (1994) in *Handbook of Natural Toxins,* Volume 8: *Microbial Toxins* (Iglewski, B., Moss, J., Tu, A.T., and Vaughan, M., eds) pp. 425-439, Marcel Dekker, New York.

4. Wolff, J., Cook, G.H., Goldhammer, A.R. and Berkowitz, S.A. (1980) *Proc. Natl. Acad. Sci.* U.S.A. **77**, 3841-3844.

5. Confer, D.L. and Eaton, J.W. (1982) *Science* **217**, 948-950.

6. Sakamoto, H., Bellalou, J., Sebo, P. and Ladant, D. (1992) *J. Biol. Chem.* **267**, 13598-13602.

7. Szabo, G., Gray, M., and Hewlett, E., (1994) *J. Biol. Chem.* **269**, 22496-22499.

8. Benz, R., Maier, E., Ladant, D., Ullmann, A. and Sebo, P., (1994), *J. Biol. Chem.* **269**, 27231-27239.

9. Glaser, P., Ladant, D., Senger, O., Pithot, F., Ullmann, A. and Danchin, A. (1988) *Mol. Microbiol.* **2**, 19-30.

10. Welch, R., (1994) *Mol. Microbiol.* **5**, 521-528.

11. Coote, J.G., (1992) *FEMS Microbiol. Rev.* **88**, 137-162.

12. Gentile, F., Knipling, L.G., Sackett, D.L. and Wolff, J. (1990) *J. Biol. Chem.* **265**, 10686-10692.

13. Hewlett, E.L., Gordon, V.M., McCaffery, J.D., Sutherland, W.M. and Gray, M.C. (1989) *J. Biol. Chem.* **264**, 19379-19384.

14. Rogel, A., Schultz, J., Brownlie, M., Coote, J., Parton, R., and Hanski, E. (1989) *EMBO J.* **8**, 2755-2760.

15. Sebo, P., Glaser, P., Sakamoto, H., and Ullmann, A. (1991) *Gene* **104**, 19-24.

16. Hackett, M., Guo, L., Shabanowitz, J., Hunt, D.F. and Hewlett, E.L. (1994) *Science* **266**, 433-435.

17. Hackett, M., Walker, C.B., Guo, L., Gray, M.C., Van Cuyk, S., Ullmann, A., Shabanowitz, J., Hunt, D.F., Hewlett, E.L. and Sebo, P. (1995) *J. Biol. Chem.* **270**, 20250-20253.

18. Hewlett, E.L., Gray, M.C., Ehrmann, I.E., Maloney, N.J., Otero, A.S., Gray, L., Allietta, M., Szabo, G. Weiss, A.A., and Barry, E.M. (1993) *J. Biol. Chem.* **268**, 7842-7848.

19. Lee, S.-L., Gray, M.C., Guo, L., Sebo, P., Hewlett, E.L. (1999) *Infect. Immun.* **67**, 2090-2095.

20. Weingart, C.L., Mobberley-Schuman, P.S., Hewlett, E.L., Gray, M.C., Weiss, A.A. (2000) *Infect. Immun.* **68**, 7152-7155.

21. Sebo, P., Bumba, L., Masin, J., Basler, M., Fiser, R., Osicka, R., Osickova, A., Adkins, I., Kamanova, J., Morova, J., Holubova, J., Kosova, M., Stanek, O., Linhartova, I. Cerny, O. Sadilkova, L., Konopasek, I., Cerny, J., Hewlett, E.L. (2010) 9th International Bordetella Symposium, Baltimore, MD, abstract.