SANTA CRUZ BIOTECHNOLOGY, INC.

Cya A (b-300): sc-33620

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The Power to Question

BACKGROUND

Bordetella pertussis, the causative agent of whooping cough, secretes several toxins implicated in this disease. One of these putative virulence factors is the adenylate cyclase toxin (Cya A or ACT), which elevates intracellular cAMP in eukaryotic cells to cytotoxic levels upon activation by endogenous calmodulin. The *Bordetella pertussis* Cya toxin-encoding locus (Cya) is composed of five genes. The Cya A gene encodes a virulence factor, Cya A, exhibiting adenylate cyclase, hemolytic and invasive activities. Cya A is related to the RTX (repeats in toxin) family of pore-forming toxins. Like all RTX toxins, Cya A is synthesized as a protoxin (proCya A) encoded by the cyaA gene. Activation to the mature cell-invasive toxin involves palmitoylation of Lysine 983 and is dependent on co-expression of Cya C. The Cya B, D and E gene products are necessary for Cya A transport, and the Cya C gene product is required to activate Cya A. Additionally, Cya A uses the $\alpha M\beta 2$ Integrin (CD11b/CD18) as a cell receptor. Thus, the cellular distribution of CD11b, mostly on neutrophils, macrophages, and dendritic and natural killer cells, supports a role for Cya A in disrupting the early, innate antibacterial immune response.

REFERENCES

1. Sebo, P., Glaser, P., Sakamoto, H. and Ullmann, A. 1991. High-level synthesis of active adenylate cyclase toxin of *Bordetella pertussis* in a reconstructed *Escherichia coli* system. Gene 104: 19-24.
2. Gross, M.K., Au, D.C., Smith, A.L. and Storm, D.R. 1992. Targeted mutations that ablate either the adenylate cyclase or hemolysin function of the bifunctional Cya A toxin of *Bordetella pertussis* abolish virulence. Proc. Natl. Acad. Sci. USA 89: 4898-4902.
3. Ehrmann, I.E., Weiss, A.A., Goodwin, M.S., Gray, M.C., Barry, E. and Hewlett, E.L. 1992. Enzymatic activity of adenylate cyclase toxin from *Bordetella pertussis* is not required for hemolysis. FEBS Lett. 304: 51-56.
4. Westrop, G.D., Hormozi, E.K., Da Costa, N.A., Parton, R. and Coote, J.G. 1996. *Bordetella pertussis* adenylate cyclase toxin: proCya A and Cya C proteins synthesised separately in *Escherichia coli* produce active toxin *in vitro*. Gene 180: 91-99.
5. Guermonprez, P., Khelef, N., Blouin, E., Rieu, P., Ricciardi-Castagnoli, P., Guiso, N., Ladant, D. and Leclerc, C. 2001. The adenylate cyclase toxin of *Bordetella pertussis* binds to target cells via the $\alpha M\beta 2$ Integrin (CD11b/CD18). J. Exp. Med. 193: 1035-1044.

SOURCE

Cya A (b-300) is a rabbit polyclonal antibody raised against amino acids 1-300 mapping at the N-terminus of Cya A of *B. pertussis* origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

Cya A (b-300) is recommended for detection of Cya A of *B. pertussis* and *B. bronchiseptica* origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1–2 µg per 100–500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Molecular Weight of Cya A: 233 kDa.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

SELECT PRODUCT CITATIONS

1. Workentine, M.L., et al. 2009. The GacS-GacA two-component regulatory system of *Pseudomonas fluorescens*: a bacterial two-hybrid analysis. FEMS Microbiol. Lett. 292: 50-56.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.