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ProductInformation

Cholera Toxin from *Vibrio cholerae*

Product Number **C 3012** Storage Temperature 2-8 °C

Product Description

CAS Number: 9012-63-9

Cholera toxin is an activator of adenylate cyclase, which causes many metabolic alterations. Tissue culture cells treated with the toxin are not killed and tissues of animals do not become necrotic. *In vivo*, cholera toxin causes edema and erythema.^{1,2} There are numerous types of cholera toxin, with their mechanisms of action varying according to type.³

A single isoelectric variant of the toxin oligomer has been isolated that crystallizes readily and reproducibly. The isoelectric homogenity gave improved crystallization behavior and features of the crystal structure have been studied.⁴ Cholera Toxin has an isoelectric point (pl) of 6.6. Chromatographic properties, however, suggest a cationic surface is exposed at pH 7.0 that apparently resides in B Subunit.⁵

Cholera toxin is one of a group of bacterial protein toxins that includes diphtheria, pertussis, shigella, tetanus, botulinum, and anthrax toxins, and Pseudomonas exotoxin A. Several bacterial toxins are ADP-ribosyltransferases with protein substrates and recent observations suggest that the enzymatic components of ADP-ribosylating toxins share some primary structure similarities. Many of the substrates ADP-ribosylated by these bacterial protein toxins are G-proteins, which are involved in signal transduction (passage of information across membranes) and ADPribosylation has been recognized as one of the more significant post translational modifications of proteins.

Cholera toxin is made up of a subunit or peptide fragment (called fragment A or A subunit), which are responsible for the enzymatic function. It is combined with one or more components (called fragment B or B subunit), which is associated with the cell surface receptor binding and subsequent internalization (transmembrane transport) of the enzymatic component. Cholera toxin is an hexameric protein composed of a single A subunit (MW 27,234) and 5 B subunits (MW 11,677 each), which are arranged as a pentameric ring with apparent 5-fold symmetry.^{6,7} The A subunit, synthesized as a single polypeptide, is proteolytically nicked during secretion from the bacterium to give rise to two disulfide-linked polypeptides, A1 (MW 21,826) and A2 (MW 5,407). It is the A1 fragment of A subunit, released by disulfide reduction, that acts enzymatically within the target cells as an ADP-ribosyltransferase. The entire oligomer (MW 85,260) is required for toxic behavior. Choleragenoid, the intact pentamer of B subunit interacts with a ganglioside GM1 membrane receptor, but cannot activate adenylyl cyclase, whereas the A subunit alone does not enter the cell.⁸

The product is lyophyllized from a solution of 1 mg of protein per ml of 50 mM Tris buffer, pH 7.5, containing 0.2 M NaCl, 1 mM sodium EDTA, and NaN₃. The amount of sodium azide in the final product is less than 1%; the exact concentration varies by lot.

Precautions and Disclaimer

For Laboratory Use Only. Not for drug, household or other uses.

Preparation Instructions

When the vial is reconstituted with 1 ml of water, the solution will contain approximately (varies by lot) 0.05 M Tris buffer pH 7.5, with 0.2 M NaCl, 3 mM sodium azide, and 1 mM sodium EDTA. EDTA is added to stabilize and preserve material during long term storage. This preparation is readily water soluble, but vigorous shaking may cause damage and loss of activity.

Solutions can be sterile filtered through a 0.2 µm filter.

Storage/Stability

Once reconstituted, the product is stable for about 1 year when stored at 2-8 °C. Solutions will lose biological activity after prolonged exposure to pH below 6 or above 8.

Cholera Toxin can be aliquoted and frozen at -20 °C. Solutions should be snap frozen (using an ethanol/dry ice bath). Repeat freeze/thaw cycles are not recommended.

References

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- 2. J. Hyg., **96**, 49 (1986).
- 3. Microbial Toxins, **2A**, (1971) p. 189.
- Spangler, B.D., and Westbrook, E.M., Crystallization of Isoelectrically Homogenous Cholera Toxin. Biochem., 28, 1333 (1989).
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- Ribi, H. O., et al., Three-dimensional Structure of Cholera Toxin Penetrating a Lipid Membrane. Science, 239(4845), 1272-1276 (1988).
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