

Product Information

Cholera Toxin B subunit from *Vibrio cholerae* biotin conjugate

Catalog Number **C9972**
Storage Temperature 2–8 °C

Product Description

Cholera toxin is the virulent factor from *Vibrio cholerae* that leads to severe diarrhea followed by dehydration in humans.^{1,2} Several bacterial toxins are ADP-ribosyl-transferases with protein substrates. Many of the substrates ADP-ribosylated by bacterial protein toxins are G-proteins, which are involved in signal transduction and ADP-ribosylation is one of the more significant post translational modifications of proteins. The ADP-ribosylation activity of cholera toxin activates adenylate cyclase, resulting in the production of cyclic AMP by adenylate cyclase, which causes many metabolic alterations.^{1,2}

Cholera toxin belongs to the AB₅-subunit family of toxins.¹ The native hexameric protein has a molecular mass of ~85 kDa and contains two subunits. It consists of a single A subunit (~27.2 kDa), responsible for the ADP-ribosylation activity, and five B subunits (~11.6 kDa each), which are arranged as a pentameric ring with an apparent 5-fold symmetry and are associated with the cell surface receptor binding and subsequent internalization (transmembrane transport) of the enzymatic component.^{3,4}

A single isoelectric variant of the cholera toxin has been isolated, which crystallizes readily and reproducibly.⁵ Cholera toxin has an isoelectric point (pI) of 6.6. Chromatographic properties, however, suggest a cationic surface is exposed at pH 7.0, which apparently resides in the B subunit.⁶

The entire hexameric complex is required for toxic behaviour. Cholera toxin, the intact pentamer of B subunits, interacts with a ganglioside G_{M1} membrane receptor, but cannot activate adenylate cyclase; whereas, the A subunit alone does not enter the cell.⁷

Due to the effect on adenylate cyclase, cholera toxin and its purified A subunit are frequently used for the study of signal transduction mechanisms. In addition, cholera toxin acts as an adjuvant through the stimulation of B lymphocytes.

The cholera toxin B subunit alone is used for track tracing in neurological research, taking advantage of G_{M1} ganglioside binding and retrograde transport. Tissue culture cells treated with cholera toxin are not killed and tissues of animals do not become necrotic.

The B subunit is non-toxic to cells and possesses no intrinsic adenylate cyclase activity. The cholera toxin B subunit (CTB) attaches to cells by binding to ganglioside G_{M1}.⁸ As a result, it has been shown to be a good label for microglial cells (due to the enrichment of ganglioside G_{M1} on their cell surface), but not for oligodendrocytes or astrocytes.⁹ The B subunit has been reported to be an excellent tracer for the study of axonal transport using immunohistochemical methods. Recently it has been widely used as a marker of membrane lipid rafts, which are membrane microdomains enriched with cholesterol and sphingolipids. These lipid rafts have an important role in cell signaling and protein trafficking.¹⁰

This product is the cholera toxin B subunit (CTB) labeled with biotin (244.3 Da). The extent of labeling is ~1 mole of biotin per mole of CTB. The conjugate is prepared using (+)-biotin N-hydroxysuccinimide ester. The biotin is conjugated to the lysine residues present in the cholera toxin B subunit. It contains ~2% unconjugated biotin.

The product was prepared and packaged using aseptic technique, and sealed under vacuum. The lyophilized powder contains ~40% protein with the balance consisting of phosphate buffer salts, EDTA, and sodium azide.

The activity is measured by ELISA using G_{M1}-coated plates, anti-rabbit CTB antibodies, and peroxidase-labeled goat anti-rabbit IgG as the second antibody. Binding saturation of 50% is achieved with 0.02–1 µg/ml of cholera toxin B subunit biotin conjugate.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Cholera toxin is soluble in water at a concentration of 10 mg/ml. Swirl bottles gently during reconstitution. Avoid vigorous pipetting of solutions that may lead to foaming. Solutions can be filtered through a 0.2 µm filter.

Storage/Stability

Store the lyophilized product at 2–8 °C. The product, as supplied, is stable 2 years when stored properly.

Store reconstituted solutions at 2–8 °C. DO NOT FREEZE.

References

1. Lencer, W.I., and Tsai, B., The intracellular voyage of cholera toxin: going retro. *Trends biochem. Sci.*, **128**, 639-645 (2003).
2. Finkelstein, R.A., and Dorner, F., Cholera enterotoxin (Cholera toxin). *Pharmac. Ther.*, **27**, 37-47 (1985).
3. Roda, L.G. *et al.*, Heterogeneity of purified cholera toxin. *Biochim. Biophys. Acta*, **492(2)**, 303-315 (1977).
4. Ribi, H.O. *et al.*, Three-dimensional structure of cholera toxin penetrating a lipid membrane. *Science*, **239(4845)**, 1272-1276 (1988).
5. Spangler, B.D., and Westbrook, E.M., Crystallization of isoelectrically homogeneous cholera toxin. *Biochem.*, **28**, 1333 (1989).
6. Mekalanos, J.J. *et al.*, *Meth. Enzymology*, **165**, 169-175 (1988).
7. Middlebrook, J.L., and Dorland, R.B., Bacterial toxins: cellular mechanisms of action. *Microbiol. Rev.*, **48**, 199 (1984).
8. Heyningen, S. Van, Cholera toxin: interaction of subunits with ganglioside G_{M1}. *Science*, **183**, 656-657 (1974).
9. Nedelkoska, L., and Benjamins, J.A., Binding of cholera toxin B subunit: a surface marker for murine microglia but not oligodendrocytes or astrocytes. *J. Neurosci. Res.*, **53**, 605-612 (1998).
10. Janes, P.W. *et al.*, Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. *J. Cell Biol.*, **147**, 447-461 (1999).

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