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Product Information

Cholera Toxin B subunit
from *Vibrio cholerae*

Catalog Number **C9903**
Storage Temperature 2–8 °C

CAS RN 131096-89-4
Synonyms: Choleragenoid, CTxB, CTB

Product Description
Cholera toxin is the virulent factor from *Vibrio cholera* 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 AB_5-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. Choleragenoid, the intact pentamer of B subunits, interacts with a ganglioside G_{M1} membrane receptor, but cannot activate adenylyl cyclase, whereas the A subunit alone does not enter the cell.[7]

Due to its 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 (CTxB) 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 CTxB are not killed and tissues of animals treated with CTxB do not become necrotic.

The B subunit is non-toxic to cells and possesses no intrinsic adenylate cyclase activity. CTxB 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. CTxB has been used as a marker of membrane lipid rafts, which are membrane microdomains enriched with cholestrol and sphingolipids. These lipid rafts have an important role in cell signaling and protein trafficking.[10]

This product is the cholera toxin B subunit (CTxB). The product was prepared and packaged using aseptic technique, and sealed under vacuum. The lyophilized powder contains ~5% protein. When reconstituted with water to a final concentration of 1 mg of CTB per mL, the solution will contain 0.05 M Tris buffer, pH 7.5, 0.2 M NaCl, 3 mM NaN_3, and 1 mM sodium EDTA.

Purity: ≥95% (SDS-PAGE)
Cholera toxin A subunit: ≤0.5% (SDS-PAGE)

The activity is measured by ELISA using GM_1-coated plates, anti-rabbit CTB primary antibodies, and peroxidase-labeled goat anti-rabbit IgG as the secondary antibody. Binding saturation of 50% is achieved with 0.05–1 μg/mL of CTB.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the 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.*, **28(12)**, 639-645 (2003).
2. Finkelstein, R.A., and Dorner, F., Cholera enterotoxin (Choleragen). *Pharmacol. Ther.*, **27(1)**, 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. *Biochemistry*, **28(3)**, 1333-1340 (1989).
6. Mekalanos, J.J., Production and purification of cholera toxin. *Meth. Enzymol.*, **165**, 169-175 (1988).
7. Middlebrook, J.L., and Dorland, R.B., Bacterial toxins: cellular mechanisms of action. *Microbiol. Rev.*, **48(3)**, 199-221 (1984).
8. van Heyningen, S., Cholera toxin: interaction of subunits with ganglioside G_{M1}. *Science*, **183(4125)**, 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(5)**, 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(2)**, 447-461 (1999).

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