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Technical Bulletin

# Cholera Toxin

From Vibrio cholerae

#### C8052

Storage Temperature 2-8 °C

CAS RN 9012-63-9

Synonyms: CTX, Cholera enterotoxin, Choleragen

## **Product Description**

Cholera toxin is the virulent factor from *Vibrio cholerae* that leads to severe diarrhea followed by dehydration in humans.<sup>1,2</sup> 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.<sup>1,2</sup>

Cholera toxin belongs to the  $AB_5$ –subunit family of toxins. $^1$  The native hexameric protein has a molecular mass of  $\sim$  85 kDa and contains two subunits. It consists of a single A subunit ( $\sim$ 27.2 kDa), responsible for the ADP-ribosylation activity, and five B subunits ( $\sim$ 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.<sup>5</sup> 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 B subunit.<sup>6</sup>

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.<sup>7</sup>

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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  $G_{\text{M1}}$ .8 As a result, it has been shown to be a good label for microglial cells (due to the enrichment of ganglioside  $G_{\text{M1}}$  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 active, native cholera toxin (composed of the A and the B subunits). It is a lyophilized powder containing ~ 5% protein (Lowry-TCA). When reconstituted with water to a final concentration of 1 mg cholera toxin per mL, the solution will contain 0.05 M Tris buffer salts, pH 7.5, 0.2 M NaCl, 3 mM NaN<sub>3</sub>, and 1 mM sodium EDTA.

Purity: ≥ 90% (SDS-PAGE)

#### Precautions and Disclaimer

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  $0.2 \mu m$  filter.

## Storage/Stability

The product was prepared and packaged using aseptic technique and sealed under vacuum. Store the lyophilized powder and reconstituted solutions at 2-8 °C.

The product, as supplied, is stable 3 years when stored properly.

Solutions are reported to be stable for 1 year when stored at 2-8 °C and will lose biological activity after prolonged exposure to pH below 6 or above 8.6

DO NOT FREEZE.

## References

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