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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<sub>5</sub>-subunit family of toxins.<sup>1</sup> 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.<sup>3,4</sup>

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>

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  $G_{M1}$ .<sup>8</sup> 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.<sup>9</sup> 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.<sup>10</sup>

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:  $\geq$  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 µm 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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