

## Product Information

### **Cholera Toxin A subunit from *Vibrio cholerae***

Catalog Number **C8180**

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.<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 the 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<sub>M1</sub> 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 A subunit, synthesized as a single polypeptide, is proteolytically cleaved during secretion from the bacterium to give rise to two disulfide-linked polypeptides, A1 (~21.8 kDa) and A2 (~5.4 kDa). It is the A1 fragment of A subunit, released by disulfide reduction, that acts enzymatically within the target cells as an ADP-ribosyltransferase.

This product is the cholera toxin A subunit. The product was prepared and packaged using aseptic technique, and sealed under vacuum. The lyophilized powder contains ~5% protein. The cholera toxin B subunit is present at ≤0.5% (SDS-PAGE). When reconstituted with water to a final concentration of 1 mg cholera toxin A subunit per ml, the solution will contain 0.05 M Tris buffer, pH 7.5, 0.2 M NaCl, 3 mM NaN<sub>3</sub>, and 1 mM sodium EDTA.

#### **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. The free A subunit precipitates in aqueous solutions at protein concentrations above 10 mg/ml and neutral pH.<sup>8</sup> DO NOT FREEZE.

### References

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7. Middlebrook, J.L., and Dorland, R.B., Bacterial toxins: cellular mechanisms of action. *Microbiol. Rev.*, **48**, 199 (1984).
8. Tayot, J.L. *et al.*, Receptor-specific large-scale purification of cholera toxin on silica beads derivatized with LysoG<sub>M1</sub> ganglioside. *Eur. J. Biochem.*, **113**, 249-258 (1981).

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