3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**Cholera Toxin B subunit from *Vibrio cholerae* peroxidase conjugate**

Catalog Number **C3741**
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 $AB_5$-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 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 cholestrol and sphingolipids. These lipid rafts have an important role in cell signaling and protein trafficking.[10]

The cholera toxin B subunit-peroxidase conjugate (CTB-HRP) has been used in many applications – as a retrograde and transganglionic tracer for pelvic primary afferents,[11] for retrograde labeling of symphathetic pre-ganglionic neurones (SPN),[12] for retrograde labeling of cells in the fastigial nuclei,[13] and for axonal and terminal labeling in the brain stem.[14] In cell signaling studies it is commonly used as a marker for membrane fractions in subcellular fractionation procedures and in floatation experiments.[15,16] CTB-HRP is particularly useful for these types of studies since it eliminates the need for use of a secondary reagent for detection. A peroxidase substrate is the only reagent necessary for detection.

This product is the cholera toxin B subunit labeled with horseradish peroxidase. The extent of CTB-HRP labeling is ~2 HRP molecules per CTB pentamer (HRP:CTB ~2:1, ~100 µg of HRP conjugated to ~45 µg of CTB). The product was prepared and packaged using aseptic technique, and sealed under vacuum.

Molecular mass: ~150 kDa (gel filtration)

HRP Activity: 10–30 pyrogallol units per vial
Activity exceeds 100 pyrogallol units per mg of HRP in the CTB-HRP conjugate.

Unit definition: One pyrogallol unit converts 1 mg of pyrogallol to purpurogallin in 20 seconds at pH 6.0 at 20 °C.

$G_{M1}$ Binding activity: 50% saturation is achieved with ~3 μg/ml of CTB-HRP.

**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**
The lyophilized powder contains 11–13% protein with the balance consisting of phosphate buffer and sodium chloride. Reconstitute with 100 μl water. Swirl the bottle gently during reconstitution. Avoid vigorous pipetting that may lead to foaming. The solutions can be filtered through a 0.2 μm filter.

The reconstituted solution contains 1 mg/ml HRP, ~0.45 mg/ml CTB, 10 mM phosphate buffer, pH 7.5, and 150 mM NaCl.

**Storage/Stability**
Store the lyophilized product at 2–8 °C. The product, as supplied, is stable for 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 (Choleragen). 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).
11. Wang, H.F. *et al*., Retrograde and transganglionic transport of horseradish peroxidase-conjugated cholera toxin B subunit, wheat germ agglutinin and isolectin B4 from *Griffonia simplicifolia* I in primary afferent neurons innervating the rat urinary bladder. Neuroscience, **87**, 275-288 (1998).
12. Ranson, R.N. *et al*., The paraventricular nucleus of the hypothalamus sends efferents to the spinal cord of the rat that closely appose sympathetic preganglionic neurones projecting to the stellate ganglion. Exp. Brain Res., **120**, 164-172 (1998).
13. Asanome, M. *et al*., Augmentation of postural muscle tone induced by the stimulation of the descending fibers in the midline area of the cerebellar white matter in the acute decerebrate cat. Neurosci. Res., **30**, 257-269 (1998).
14. Robertson, B. *et al*., WGA-HRP and choleragenoid-HRP as anterogradely transported tracers in vagal visceral afferents and binding of WGA and choleragenoid to nodose ganglion neurons in rodents. Brain Res., **590**, 207-212 (1992).
15. Hawari, F.I. *et al*., Release of full-length 55-kDa TNF receptor 1 in exosome-like vesicles: a mechanism for generation of soluble cytokine receptors. Proc. Natl. Acad. Sci. USA, **101**, 1297-1302 (2004).
16. Becher, A. *et al*., The γ-aminobutyric acid receptor B, but not the metabotropic glutamate receptor type-1, associates with lipid rafts in the rat cerebellum. J. Neurochem., **79**, 787-795 (2001).


EM,ESS,NDH,EB,AH,PHC,MAM 05/11-1