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ProductInformation

Cholera Toxin B Subunit, Peroxidase from *Vibrio cholerae*

Product Number **C4672**
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] Several bacterial toxins are ADP-ribosyltransferases with protein substrates. Many of the substrates ADP-ribosylated by bacterial protein toxins are G-proteins, which are involved in signal transduction (passage of information across membranes) 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.

The native hexameric protein has a molecular weight of ~85 kDa and contains two subunits.[2,3] It consists of a single A subunit (MW ~27.2 kDa), responsible for the ADP-ribosylation activity, and five B subunits (MW ~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.[4,5]

A single isoelectric variant of the cholera toxin has been isolated, which crystallizes readily and reproducibly.[6] 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.[7]

The entire hexameric complex is required for toxic behaviour. Choleragenoid, the intact pentamer of B subunits, interacts with a ganglioside GM_1 membrane receptor, but cannot activate adenylyl cyclase; whereas, the A subunit alone does not enter the cell.[8]

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 GM_1 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 attaches to cells by binding to ganglioside GM_1.[9] As a result, it has been shown to be a good label for microglial cells (due to the enrichment of ganglioside GM_1 on their cell surface), but not for oligodendrocytes or astrocytes.[10] The B subunit has been reported to be an excellent tracer for the study of axonal transport using immunohistochemical methods. The peroxidase conjugated subunit 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. Cholera toxin B subunit conjugated to peroxidase (CB-HRP) has been shown to be a more effective retrograde and transganglionic tracer for pelvic primary afferents from the urinary bladder than wheat germ agglutinin-horseradish peroxidase or isolectin B4-horseradish peroxidase.[11] Retrograde labeling of symphathetic pre-ganglionic neurons with CB-HRP, as well as cells in the fastigial nuclei, has been performed.[12,13] It has also been shown to be an effective anterograde tracer for the study of axonal and terminal labeling in brain stem.[14]

This product is the cholera toxin B subunit labeled with horseraddish peroxidase (CTB-HRP MW = ~150 kDa). The extent of labeling is 2 HRP molecules per CTB pentamer (HRP:CTB = ~2:1).

It is a lyophilyzed powder containing 11-13% protein with the balance consisting of phosphate buffer and sodium chloride. Reconstitution of the vial with 100 µl of water will yield a final solution containing: 1 mg/ml HRP, 0.45 mg/ml CTB, 10 mM phosphate buffer and 150 mM NaCl.

HRP activity exceedes 100 pyrogallol units per mg of HRP in the CTB-HRP conjugate.

Activity: 10-30 pyrogallol units per vial.

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

Binding saturation of 50% 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
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 sterile 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 2 years when stored properly.

Solutions are 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. DO NOT FREEZE.

References

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NDH/MAM 09/04