ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Life Technologies™ Your Molecular & Cell Technology Partner

Cholera Toxin Protein

Cat. CTOX15-N-500 Purified Cholera Toxin protein (antigen grade) **Size**: ☐ 500 ug

Cholera toxin (sometimes abbreviated to CTX, Ctx, or CT) is a protein complex secreted by the bacterium Vibrio cholerae. CTX is responsible for the harmful effects of cholera infection. The cholera toxin is an oligomeric complex made up of six protein subunits: a single copy of the A subunit (part A), and five copies of the B subunit (part B). The two parts are connected by a disulfide bond. The three-dimensional structure of the toxin was determined using X-ray crystallography by Zhang et al. in 1995. The five B subunits—each weighing 12 kDa, and all coloured blue in the accompanying figure—form a five-membered ring. The A subunit has two important segments. The A1 portion of the chain (CTA1, red) is a globular enzyme payload that ADP-ribosylates G proteins, while the A2 chain (CTA2, orange) forms an extended alpha helix which seats snugly in the central pore of the B subunit ring.
This structure is similar in shape, mechanism, and sequence to the heat-labile enterotoxin secreted by some strains of the Escherichia coli bacterium.

The gene encoding the cholera toxin is introduced into V. cholerae by horizontal gene transfer. Virulent strains of V. cholerae carry a variant of lysogenic bacteriophage called CTXf or CTXφ. Once secreted, the B subunit ring of CTX will bind to GM1 gangliosides on the surface of the host's cells. After binding takes place, the entire CTX complex is internalised by the cell and the CTA1 chain is released by the reduction of a disulfide bridge. CTA1 is then free to bind with a human partner protein called ADP-ribosylation factor 6 (Arf6); binding to Arf6 drives a change in the conformation (the shape) of CTA1 which exposes its active site and enables its catalytic activity. The CTA1 fragment catalyses ADP ribosylation from NAD to the regulatory component of adenylate cyclase, thereby activating it. Increased adenylate cyclase activity increases cyclic AMP (cAMP) synthesis causing massive fluid and electrolyte efflux, resulting in diarrhea. The pentameric part B of the toxin molecule binds to the surface of the intestinal epithelium cells. Part A detaches from the pentameric part upon binding, and gets inside the cell via receptor-mediated endocytosis. Once inside the cell, it permanently ribosylates the Gs alpha subunit of the heterotrimeric G protein resulting in constitutive cAMP production. This in turn leads to secretion of H2O, Na+, K+, Cl-, and HCO3- into the lumen of the small intestine resulting in rapid dehydration.

Source of Antigen

Cholera toxin is isolated from Vibrio cholerae type Inaba 569B and it is highly purified (single major band under non-reducing conditions). It contains only trace amounts of B subunit, a by-product of lyophilization. When equal weights are compared, the A subunit exhibits 3 to 5 times the transferase activity of the holotoxin. Cholera toxin and native subunits all undergo treatment for the removal of contaminating Endotoxin. The toxin is supplied as a lyophilized powder in a buffer (0.05M Tris, 0.2M NaCl, 1 mM EDTA, ph 7.5). Reconstitute powder in water and it may become little cloudy due to decreases in solubility. Store powder or liquid at -20oC or below for 6-12 months. Suitable preservatives (azide or merthiolate may be added if necessary).

Activity:
Biological activity is tested by hemagglutination assay using trisialoganglioside fixed sheep red blood cells.

Stability: 6-12 months at –20oC or below.

Recommended Usage

Purified protein can be used for ELISA, Western, antibody titration or as control protein for adjuvant.

General References: Sato Y (1983) Infect. Immun. 41, 313-320; Spangler BD (1992) Microbiol. Rev. 56, 622-647; Gill DM (1976) Bicohem. 15, 1242; Lai CY (1977) JBC 252, 7249; Rappaport RS (1974) Infect. Immun. 9, 294-303.

This product is for in vitro research use only.

Related material available from ADI

Diphteria Toxoid, Toxin (whole), subunits A and B

Antibodies to subunits A and B

ELISA Kits for the detection of anti-Diphterai Toxoid IgG in rabbit, mouse, and human.

CTOX15-N-500 110825A

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com