Clostridium difficile Toxins
TOXIN A, TOXIN B
TOXIN A AND TOXIN B TOXOIDS
BINARY TOXIN

Clostridium difficile is the causative agent of antibiotic-associated diarrhea and pseudomembranous colitis.[1,2] It produces two major exotoxins (Toxin A and Toxin B), which are the prototypes of the family of large clostridial cytotoxins. Some strains of *C. difficile* also produce a binary ADP-ribosylating toxin (CDT) which modifies actin.[3] The pathogenic role of CDT Binary Toxin in diseases induced by *C. difficile* is not clear, but about 10% of strains isolated from patients with colitis contain CDT Binary Toxin genes.[4,5]

Clostridium difficile A and B are high molecular weight glucosyltransferases that inhibit members of the Rho family of GTPases. Toxin A, referred to as an enterotoxin, has a molecular weight of 300 kDa and an isoelectric point, pI, of 5.3. Toxin B, known as a cytotoxin, has a molecular weight of 270 kDa and a pI of 4.1.

Both Toxin A and Toxin B deactivate small GTPases such as Rho, Rac and Cdc42 by glucosylation of a threonine residue.[6,7,8] Inhibition of these GTPases causes the shutdown of signal transduction cascades leading to depolymerization of the cytoskeleton, gene transcription of certain stress-activated protein kinases, a drop in synthesis of phosphatidylinositol 4, 5 bisphosphate and possibly even the loss of cell polarity.[9] Loss of cytoskeletal structure results in cell rounding, and this loss of structure may account for the host's reaction to *C. difficile*.

The GTPases targeted by Toxins A and B are linked to many cell responses; however, these toxins have different physiological effects. Both toxins cause irritation to skin and increased vascular permeability.[10] Only Toxin A has been shown to have a binding site on epithelial cell surface carbohydrates[11] which may be linked to a dramatic reaction seen in rabbit ileum.[23,13] Toxin B is a potent cytotoxin for mammalian cells in tissue culture.[14,15] Toxin B is at least 1,000 times more cytotoxic than Toxin A in cell rounding assays.

By inhibition of signal transduction cascades, Toxin A and Toxin B are able to block downstream responses. Toxin A has been shown to cause induction of interleukin 8.[16] Toxin B may block serotonin release,[17] as well as stimulate production of phospholipases C and D.[18,19] By virtue of its ability to affect depolymerization of the cytoskeleton, Toxin B has been shown to inhibit biphasic muscle contraction.[20]

C. difficile Toxins A and B are 63% homologous in amino acid content and have a similar three dimensional structure.[21] The C-terminal third of each toxin is made up of sequences called clostridial repetitive oligopeptides (CROPs) which are highly antigenic. The remaining N-terminal two-thirds of Toxins A and B are less similar to each other with respect to sequence homology however, it is this portion of each protein which contains the glucosyltransferase activity.[22, 23]

CDT Binary Toxin is a member of the ADP-ribosyltransferase family (ADPRT).[3] CDT Binary Toxin belongs in the AB Binary Toxin subfamily along with C2 toxin from *C. botulinum* and Iota toxin from *C. perfringens*.[24] The Binary Toxin is composed of two independently produced components, the enzymatic component A subunit, CdtA (48kD), and the binding and translocation component B subunit, CdtB (94kD), which mediates cell entry of CdtA. [25] The CDT Binary Toxin causes depolymerization of the actin cytoskeleton and formation of microtubule-based membrane protrusions, which are suggested to be involved in enhanced bacterial adhesion and colonization of hypervirulent *C. difficile* strains.[26]

The CDT Binary Toxin, B subunit, CdtB, is essential for the entry of CdtA into the cytosol.[27] The CdtB component must be activated via cleavage, after which it can form heptamers at the cell surface and bind to specific cell-surface receptors. The cell surface receptor has been identified as lipolysis stimulated lipoprotein receptor (LSR).[28] Next, CdtA binds to CdtB and is taken up into the cell by receptor-mediated endocytosis. The N-terminus of CdtA is responsible for interaction with CdtB, whereas the C-terminus contains the enzymatic activity.[29] Based on the activity of Iota toxin, it is predicted that CDT will irreversibly ADP-ribosylate monomeric G-actin at the Arg^{177} residue. This ADP-ribosylation will block polymerization of G-actin to F-actin and disrupt the F-actin:G-actin equilibrium which results in cell rounding and cell death.[30]

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LIST BIOLOGICAL LABORATORIES, INC.

List Biological Laboratories, Inc. provides highly purified native *C. difficile* Toxin A and Toxin B, and recombinant Binary Toxin, A and B subunits. Toxoids of Toxin A and Toxin B prepared by formaldehyde inactivation are also available.

List Labs has several antibodies generated to these *C. difficile* products including goat antisera to *C. difficile* Toxin A as well as affinity purified chicken IgY to both *C. difficile* Toxin A and Toxin B, and the *C. difficile* Binary Toxin A and B subunits. The Anti-Binary B antibody is available both with and without biotinylation.

These products are intended for research purposes and are not intended for use in humans or as diagnostic agents. For further information, please contact List Biological Laboratories, Inc.

Ordering Information

Product No.	Description	Size
152C	Toxin A from *C. difficile*	100 µg
153	Toxin A Toxoid from *C.difficile*	100 µg
154A	Toxin B Toxoid from *C. difficile*	20 µg
155A,B	Toxin B from *C. difficile*	2 µg, 20 µg
155L	Toxin B from *C. difficile,* Liquid	50 µg
157A	Binary Toxin, A Subunit	20 µg
158A	Binary Toxin, B Subunit	40 µg
752	Anti-Toxin A from *C. difficile* (Goat)	0.5 ml
753A	Anti-*Clostridium difficile* Toxin A (Chicken IgY), Liquid	0.1 mg
754A	Anti-*Clostridium difficile* Toxin B (Chicken IgY), Liquid	0.1 mg
757A	Anti-*Clostridium difficile* Binary Toxin, A Subunit (Chicken IgY), Liquid	0.1 mg
758A	Anti-*Clostridium difficile* Binary Toxin, B Subunit (Chicken IgY), Liquid	0.1 mg
759A	Biotinylated Anti-*Clostridium difficile* Binary Toxin, B Subunit (Chicken IgY), Liquid	0.1 mg

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