BOTULINUM NEUROTOXINS

Clostridium botulinum neurotoxins (BoNTs) block the release of acetylcholine from peripheral cholinergic nerve endings[1] causing botulism in man and animals.[2,3] Seven immunologically distinct serotypes of neurotoxin, designated types A through G, have been identified.[4] Each is synthesized as a single polypeptide chain (MW 150,000), the holotoxin.[5] These holotoxins become embedded in a complex formed from hemagglutinins and non-hemagglutinating proteins, which enhance oral toxicity by stabilizing the neurotoxins.[1] When botulinum neurotoxin is exposed to proteases, either during cultivation or subsequent to purification of the toxin, specific peptide bonds are cleaved or "nicked" resulting in the formation of a dichain molecule.[4,6] Dichain neurotoxin is composed of a light chain region (MW 50,000) linked by a single disulfide bond and non-covalent interactions to a heavy chain (MW 100,000).[7] Conversion of the single chain form of a neurotoxin to its dichain form generally results in increased toxicity.[7] When the light and heavy chains of botulinum toxin are separated from one another, neither is capable of blocking neurotransmitter release in unaltered cells. However, the light chain alone is capable of blocking acetylcholine release if injected directly into the cell cytosol.[8,9]

All serotypes of botulinum neurotoxin are zinc-dependent proteases. Enzymatic activity resides exclusively in the light chain of the molecules. These enzymes cleave SNARE proteins (synaptobrevin-2, syntaxin or SNAP-25), which form the core of a complex involved in the fusion of transmitter-containing vesicles with the plasma membrane.[10] Prior to fusion, the SNARE proteins in the vesicle and plasma membrane interact forming a complex which contracts with an increase in the intracellular calcium concentration, pulling the vesicle close to the plasma membrane. Interaction between lipids in the two membranes allows the vesicle and nerve terminal active zone to fuse.[13,14] During this fusion, the contents of the vesicles, mainly neurotransmitters, are released, and the inner surface of the vesicles are exposed to the synaptic cleft. If one of the SNARE proteins is cleaved by a neurotoxin, SNARE complex formation cannot occur and fusion is interrupted. Botulinum toxins type B, D, F and G cleave synaptobrevin-2 (MW 19,000) which is located in vesicular membranes.[10] Syntaxin (MW 36,000) and SNAP-25 (MW 25,000) are attached to the inner surface of the plasma membrane in nerve endings close to the active zone. Syntaxin is cleaved by botulinum neurotoxin type C1, and SNAP 25 by botulinum neurotoxins type A, C1 and E.[15,16,17]

Blockage of acetylcholine release from nerve endings by botulinum toxin proceeds through a multi-step process that includes binding, receptor-mediated internalization, translocation across a membrane, reduction and proteolysis of substrates.[11,12] Toxins must pass through the plasma membrane of nerve cells to gain access to their intracellular targets. A thirty-four amino acid sequence on the C-terminal of both botulinum toxins type A and type B binds to specific types of gangliosides with low affinity.[1] In addition, a motif within the C-terminal half of the heavy chain is thought to bind to a protein, representing the high affinity binding site.[10] In the case of BoNT/B, synaptotagmin, a protein spanning the vesicular membrane, was shown to function as the receptor.[18,26] The protein receptor for BoNT/A has been identified as the SV2 protein.[27,28] Presentation of the binding protein allows the toxin to attach to the membrane. Following endocytosis the protein-bound toxin molecules are trapped in vesicles, where the contents are acidified by an ATP-driven proton carrier.[19] The N-terminal half of the heavy chain may undergo conformational changes at low pH allowing its insertion into and penetration through the endosomal membrane.[20] As neurotoxin enters the cytosol, the disulfide link between light and heavy chains is reduced.[7,21] At this point, light chains are active and able to cleave SNARE proteins which are not complexed. This multistep process is similar to the intoxication with tetanus toxin, another closely related clostridial neurotoxin.[1,22]

Botulinum neurotoxins are valuable research tools in studies aimed at elucidating the mechanisms involved in vesicle trafficking, and in gaining an understanding of the underlying events of synaptic transmission.[23,24] Botulinum neurotoxins are the most deadly bacterial toxins known because of their ability to cause cessation of neurotransmitter release at the neuromuscular junction and autonomic nerve endings leading to disturbances as well as to fatal paralysis. Ironically, it is this property of botulinum toxin which has been successfully harnessed and used clinically to treat certain neuromuscular disorders in humans, such as blepharospasm, strabismus, and torticollis.[25]

List Biological Laboratories, Inc. (List Labs) provides highly purified preparations of botulinum neurotoxin and complex from *Clostridium botulinum* types A, B, D and E. Botulinum neurotoxin Type A is "nicked" during cultivation. Serotype A is also provided with more testing and documentation as QD grade Botulinum Toxin Type A. Both the complex and holotoxin for the Type B and E are nicked after purification and are available as the activated forms. The Type D neurotoxin is recombinantly expressed in, and purified from, *E. coli* and is also activated by nicking. Toxoids derived from botulinum neurotoxin types A and B are also available. In addition, List Labs offers recombinantly produced light chains from types A, B, C, D, E and F. These light chains are non-toxic proteins that retain the enzymatic activity encoded by the holotoxin. They lack any binding domain and are unable to gain access to intracellular targets without microinjection. Light chain from types A, C and E are capable of cleaving the eukaryotic substrate SNAP-25 (**#500A**). Light chain A is also highly active with the quenched fluorogenic peptide substrates, SNAPtide® (**#520, 521** and **523**), developed by List Labs. The light chains from type B, D and F are capable of cleaving the eukaryotic substrate synaptobrevin-2 (**#510A**). Light chain from type B also efficiently cleaves the quenched fluorogenic peptide substrates, VAMPtide® (**#540**, **541** and **542**). We have available SNAP Etide® (**#550**), a quenched fluorogenic substrate for light chain type E and SYNTAXtide® (**#560**) a fluorogenic substrate for light chain type C. Heavy chain binding domains for serotypes A and B are available with and without a GST tag. A GST fusion protein containing the luminal domain loop of SV2c is also available, **#690B** (U.S. Patent #8,476,024).[26,27,28] IgY antibodies against the heavy chain binding domain of botulinum neurotoxins types A and B and a mouse monoclonal to botulinum neurotoxin type A light chain are available (**#730**, **731**, and **736**). **These products are intended for research purposes only and are not intended for use in humans or as diagnostic agents. For further information, please contact List Biological Laboratories, Inc.**

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LIST BIOLOGICAL LABORATORIES, INC.

Ordering Information

Product No.	Description	Sizes
	***Clostridium Botulinum* Neurotoxins**	
128 A,C	Botulinum Neurotoxin Type A Complex	10 µg, 100 µg
130 A,B	Botulinum Neurotoxin Type A	10 µg, 100 µg
9130A	QD grade Botulinum Neurotoxin Type A	10 µg
133L	Botulinum Neurotoxin Type A Toxoid, Liquid	10 µg
136 A,B	Botulinum Neurotoxin Type B, Nicked	10 µg, 100 µg
138 A,B	Botulinum Neurotoxin Type B Complex, Nicked	10 µg, 100 µg
139	Botulinum Neurotoxin Type B Toxoid	10 µg
140 A,B	Botulinum Neurotoxin Type E Complex, Nicked	10 µg, 100 µg
141A	Botulinum Neurotoxin Type E, Nicked	10 µg
146A	Botulinum Neurotoxin Type D, Nicked, Recombinant	10 µg
	***Clostridium Botulinum* Neurotoxin Native Substrates**	
500A	SNAP-25 Recombinant Protein Substrate for *C. botulinum* Types A and E Neurotoxin	100 µg
510A	GST Synaptobrevin-2 Recombinant Protein Substrate for *C. botulinum* Types B, D, F and tetanus toxin	100 µg
	***Clostridium Botulinum* Neurotoxin FRET Peptide Substrates**	
	FRET peptide substrates for *C. botulinum* Type A neurotoxin	
520	SNAPtide® (oAbz/Dnp) Peptide Substrate	200 nmoles
521	SNAPtide® (FITC/DABCYL) Peptide Substrate	200 nmoles
523	SNAPtide® flP6 (DABCYL/5-IAF) Peptide Substrate	200 nmoles
	FRET peptide substrate for *C. botulinum* Type B neurotoxin	
540	VAMPtide ® (oAbz/Dnp) Peptide Substrate	200 nmoles
541	VAMPtide ® (FITC/DABCYL) Peptide Substrate	200 nmoles
542	VAMPtide ® (PL 150, Pya/Nop) Peptide Substrate	200 nmoles
	FRET peptide substrate for *C. botulinum* Type E neurotoxin	
550	SNAP Etide® (oAbz/Dnp) Peptide Substrate	100 nmoles
	FRET peptide substrate for *C. botulinum* Type C neurotoxin	
560	SYNTAXtide® (oAbz/Dnp) Peptide Substrate	200 nmoles
	Recombinant Light and Heavy Chains from *Clostridium Botulinum* Neurotoxins	
	Recombinant Light Chains	
610A	Type A Light Chain	10 µg
611A	Type A Light Chain, GST fusion	15 µg
620A	Type B Light Chain	10 µg
625A	Type C Light Chain	10 µg
630A	Type D Light Chain	10 µg
635A	Type E Light Chain	10 µg
640 A,B	Type F Light Chain	10 µg, 100 µg
	Recombinant Heavy Chains	
612A	Type A Heavy Chain Binding Domain	50 µg
613A	Type A Heavy Chain Binding Domain, GST fusion	50 µg
622A	Type B Heavy Chain Binding Domain	50 µg
623A	Type B Heavy Chain Binding Domain, GST fusion	50 µg
	Antibodies	
730A	Anti-Botulinum Neurotoxin Type A, IgY from chicken Raised against the heavy chain binding domain of botulinum Type A neurotoxin, Product #612	0.1 mg/ml
731L	Anti-Botulinum Neurotoxin Type A (mouse IgG monoclonal F1-40) Monoclonal antibody binds the light chain of botulinum neurotoxin Type A	0.1 mg/ml
736A	Anti-Botulinum Neurotoxin Type B, IgY from chicken Raised against the heavy chain binding domain of botulinum Type B neurotoxin, Product #622	0.1 mg/ml
	Receptor	
690B	GST-SV2c, Receptor for Botulinum Neurotoxin Type A Luminal domain loop of SV2c (U.S. Patent #8,476,024)	100 µg

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