TETANUS TOXIN, TETANUS TOXOID, TETANUS LIGHT CHAIN, C-FRAGMENT, FITC C-FRAGMENT AND GST SYNAPTOBREVIN 2

Tetanus toxin is a potent neurotoxin (150,000 Da) produced by the anaerobic bacterium *Clostridium tetani*.[1] Intact toxin derived from culture supernatants consists of two polypeptide chains connected through an interchain disulfide bond.[2,3] The larger of the two polypeptides, heavy chain (100,000 Da), contains the toxin's binding and translocation domains. The smaller of the two polypeptides, light chain (50,000 Da), is a protease which cleaves synaptobrevin 2, also known as VAMP 2.[1] Synaptobrevin 2 is a presynaptic, vesicle membrane associated protein that, in the presence of calcium, forms a stable complex with two plasma membrane attached proteins, syntaxin and SNAP25.[2] Complex formation initiates fusion of transmitter containing vesicles with the plasma membrane. If synaptobrevin 2 is cleaved by tetanus toxin, fusion is prevented, and neurotransmitter release is inhibited. Tetanus toxin preferentially blocks release of the inhibitory transmitters, glycine and GABA, when the toxin reaches the inhibitory neurons.

The unique behavior of tetanus toxin is responsible for its specificity *in vivo*. It first binds to gangliosides on peripheral nerve endings and is internalized through receptor-mediated endocytosis. Toxin travels to the ventral horn by axoplasmic transport.[4,5] From there it is released into the interneuronal space and is subsequently taken up by the inhibitory interneurons adjacent to the soma of the motoneurons.[6] Once internalized into vesicles, it undergoes pH-dependent conformational changes leading to translocation through endosomal membranes into the cytosol.[7] In the cytosol, the light chain is primed by reducing enzymes like thioredoxin reductase, which separate the light chain from the heavy chain.[8] Light chain, the activated protease, cleaves synaptobrevin 2, blocking exocytosis of inhibitory transmitters. Consequently, incoming excitatory inputs from upstream are passed on, unfiltered, downstream. In addition to activity in the motor system, tetanus toxin is also taken up by nerve endings of sensory and vegetative neurons.[9,10,11]

When tetanus toxin undergoes mild enzymatic cleavage with papain, two major polypeptides are generated.[11,12] The larger polypeptide (~100,000 Da) consists of the N-terminal portion of the heavy chain, which contains the pH-dependent translocation domain, and the light chain linked by a disulphide bond. The smaller polypeptide (~50,000 Da), known as the C-fragment, consists of the C-terminal or binding portion of the heavy chain. The C-fragment retains the binding activity and internalization properties associated with the intact tetanus toxin.[13] It has the advantage of being non-toxic and virtually devoid of any action on the nerve processes in which it is transported. This unique combination of properties makes products such as C-fragment extremely useful for tracing fiber connections in the CNS. Using immunohistochemical techniques, it has been possible to demonstrate transsynaptic retrograde transport of unlabeled C-fragment.[13] Conjugates of C-fragment have been shown to undergo extensive transfer from motoneurons to presynaptic terminals in the spinal cord,[13] and a fluorescein-labeled conjugate of C-fragment has been utilized in a developmental study of mouse neuromuscular junctions.

Tetanus toxin from List Biological Laboratories, Inc. is prepared from cultures of *Clostridium tetani.* In SDS-gel electrophoresis, the intact toxin migrates as a single band (150,000 Da). Upon reduction, the toxin migrates as two bands (100,000 and 50,000 Da) corresponding to the heavy and light chains, respectively. Tetanus toxin, in the reduced form, cleaves recombinant GST synaptobrevin 2 in *in vitro* assays.

Tetanus toxoid is prepared by formaldehyde inactivation of pure neurotoxin. Each lot is verified to be non-toxic. In addition to being a potent antigen, tetanus toxoid is a useful carrier protein for making polysaccharides and haptens immunogenic.[14,15]

Tetanus toxin C-fragment is manufactured by enzyme digestion of native tetanus toxin produced in *Clostridium tetani* cultures. Each lot of C-fragment is tested for binding activity to G_{T1b} ganglioside. The FITC conjugate of C-fragment (C-FITC) also exhibits G_{T1b} ganglioside binding activity.

Tetanus light chain is manufactured as a recombinant protein. Each lot of tetanus light chain is tested for enzymatic activity in an endopeptidase reaction with the recombinant eukaryotic substrate GST synaptobrevin 2. Tetanus light chain is a non-toxic protein that retains the enzymatic activity encoded by the holotoxin. With this product there is no chance of contamination by heavy chain since only the light chain is encoded and expressed. Light chain is unable to gain access to intracellular targets without microinjection.

Tetanus toxin is one of the most potent toxins known to man. Extreme care should be exercised in its use. It is recommended that all laboratory personnel handling this product have a current vaccination and a serum antitoxin titer exceeding 1.0 international unit per milliliter. Toxoid, C-fragment and its conjugate are derived from tetanus toxin. Due to the sometimes unavoidable presence of trace amounts of intact toxin in these products, it is recommended that the precautions described for intact toxin be followed when using these derivatives. Recombinant light chain is considered non-toxic because there is no chance of contamination by heavy chain or intact toxin.

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Ordering Information

Product No.	Description	Size
190A,B	Tetanus Toxin	25, 100 µg
191A,B	Tetanus Toxoid	25, 100 µg
193	Tetanus Toxin C-Fragment	10 µg
196A	Tetanus Toxin C-Fragment-Fluorescein Isothiocyanate Conjugate	10 µg
510A	GST Synaptobrevin 2, Recombinant Protein Substrate	100 µg
650A	Tetanus Neurotoxin Light Chain, Recombinant	10 µg

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