

DIPHTHERIA TOXIN DIPHTHERIA TOXOID CRM MUTANT

Diphtheria toxin, secreted by certain strains of *Corynebacterium diphtheriae*, catalyzes the ADP-ribosylation of eukaryotic aminoacyl-transferase II (EF-2) using NAD as a substrate.^{1,2} This reaction forms the basis for its toxicity toward eukaryotic organisms.^{3,4,5}

Diphtheria toxin is synthesized and excreted as a proenzyme, composed of a single polypeptide chain having an approximate molecular weight of 58,000 daltons.^{6,7} For its enzymatic activity to be expressed, the toxin must undergo two covalent alterations in structure. First, mild proteolysis results in the formation of "nicked toxin," which is enzymatically inactive and consists of two major fragments, A and B, linked by a disulfide bond. Reduction of the nicked toxin with thiols releases the N-terminal A fragment (molecular weight 21,000 daltons) which is enzymatically active. The C-terminal B fragment (molecular weight 37,000 daltons) has no apparent enzymatic activity, but is required for toxicity. Evidence suggests that the B fragment is responsible for recognizing and binding the toxin to cell surface receptors.^{8,9,10}

Diphtheria toxin receptor has been shown to be a cell-surface expressed heparin-binding epidermal growth factor-like growth factor (HB-EGF) precursor.¹¹ In monkey cells, another membrane protein, CD-9 increases the affinity of diphtheria toxin for HB-EGF, indicating that two proteins may function together as a receptor.^{12,13} Sensitivity of mammalian cell lines to diphtheria toxin varies with the presence of receptors. Monkey Vero cells are highly sensitive,¹⁴ hamster cells moderately sensitive, where rat and mouse cells without receptors are resistant.¹⁵ Reviews of the literature on diphtheria toxin have been written by both R.K. Holmes¹⁶ and R.J. Collier.¹⁷

Since 1983, List Biological Laboratories, Inc. has offered pure diphtheria toxin, from *C. diphtheriae* Park Williams strain 8, which is purified by a modification of the method of Pappenheimer *et al.*¹⁸ As assessed by SDS gel electrophoresis run in the presence of a reducing agent, it migrates as a major band with an approximate molecular weight of 58,000 daltons corresponding to the intact toxin, and two smaller more lightly stained bands of approximate molecular weights 37,000 and 21,000 daltons, corresponding to the B and A fragments, respectively. By this method, the extent of nicking in a typical preparation is estimated to be less than 5%. Following trypsin treatment and reduction with dithiothreitol, diphtheria toxin from List Biological Laboratories, Inc. exhibits high specific activity. Diphtheria toxin is active on CHO¹⁹ or Vero²⁰ cells when diluted to a nanogram or less per milliliter.

Mutant forms of diphtheria toxin (DT), isolated in the early 1970s, contributed to an understanding of the AB structure of DT.¹⁷ Uchida *et al* described the isolation and properties of several mutants including cross-reactive material (CRM) 197.^{21,22} CRM 197 is a non-toxic DT mutant containing a lesion in the A chain blocking ADP-ribosylation. CRM results from a single base change in the structural gene resulting in the substitution of glutamic acid for glycine. While CRM shows no enzymatic activity, it is immunologically indistinguishable from diphtheria toxin.¹⁸

In its applications, CRM 197 is similar to diphtheria toxoid. CRM has the advantage of being a well defined protein in contrast to formaldehyde treated toxin (toxoid) which is non-specifically cross linked and subject to rearrangement.^{23,24} CRM functions as a carrier for polysaccharides and haptens making them immunogenic.^{25,26,27} On SDS gels, this protein migrates as a single major band with an approximate molecular weight of 58,000 daltons.

Diphtheria toxoid, prepared by formaldehyde inactivation of diphtheria toxin, is also available. Diphtheria toxin, CRM 197, and toxoid are supplied as aseptically prepared lyophilized powders, stoppered under vacuum. Refer to the lot specific Certificate of Analysis for buffer formulation and vial content.

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

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ORDERING INFORMATION

Product No.	Description	Size
149	Diphtheria Toxin CRM Mutant	0.5 mg
150	Diphtheria Toxin (unnicked)	1 mg
151	Diphtheria Toxoid	0.5 mg

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