

## PERTUSSIS TOXIN from *Bordetella pertussis*

Pertussis toxin (PTX) is produced by *Bordetella pertussis*, the bacterium responsible for whooping cough. Pertussis toxin is a multi-component protein composed of six non-covalently bound subunits ranging in molecular weight from approximately 9 to 28 kDa. These subunits are designated as S1, S2, S3, S4 and S5 and occur in native pertussis toxin in a ratio of 1:1:1:2:1, where the subunit S4 is present in two copies.<sup>1,2</sup> The largest subunit S1, also called the A protomer, is responsible for the ADP-ribosyltransferase activity; the A protomer alone will transfer the ADP ribose from NAD<sup>+</sup> to  $\alpha$  subunits of G proteins of the class G $\alpha_i$ , G $\alpha_o$  or G $\alpha_t$ . The crystal structure of PTX reveals a pyramid-like shape with the A protomer situated on top of the S5 subunit, which rests on two dimers, S2-S4 and S3-S4.<sup>3</sup> Together the five subunit platform is called the B oligomer, and under certain conditions PTX dissociates into just two parts, the enzymatic A protomer and the five subunit binding complex, the B oligomer. This B oligomer allows PTX to enter most cells, attaching to glycan residues present on receptor proteins including TLR4<sup>4</sup> and glycoprotein Ib.<sup>5</sup> After entering the cell via receptor-mediated endocytosis, PTX is transported retrogradely via the endosomal pathway and Golgi complex to the endoplasmic reticulum. A protomer is released from the toxin and translocates through the membrane of the endoplasmic reticulum where the toxin inactivates the target membrane-bound G proteins.<sup>6,7</sup>

In mammalian cells, G proteins serve to keep cellular adenylate cyclase in check. Within these cells, pertussis toxin catalyzes the breakdown of cellular NAD<sup>+</sup>, transferring the ADP-ribose moiety to alpha subunits of G proteins of the heterotrimeric G<sub>i/o</sub> protein subfamily. The covalent modification of G proteins disrupts signaling pathways where G protein binding is part of the chain of events.<sup>8,9</sup> When a signaling molecule binds to a G protein-coupled receptor on the surface of a pertussis-intoxicated cell, G protein is not available to pass on the message to intracellular effectors. Thus, ADP-ribosylated G proteins can no longer inhibit the cellular adenylate cyclase, allowing production of cyclic AMP, without restraint. Since cAMP is a key signaling molecule, accumulation of cAMP as a result of PTX intoxication stimulates cAMP mediated pathways. Many of the effects of pertussis toxin are due to this process.

Also, since G proteins are present in several mammalian cell types, pertussis toxin affects most cultured cells. In cell biology, pertussis toxin is useful in eliminating and thereby identifying G<sub>i/o</sub> protein-dependent pathways. G<sub>i/o</sub> protein-dependent effects of pertussis toxin are numerous and include lymphocytosis,<sup>10,11</sup> histamine sensitization<sup>12</sup> and insulin secretion which is reviewed by Straub *et al.*<sup>13</sup> Additionally, pertussis toxin appears to act through a phosphokinase C pathway to increase the permeability of the blood-brain barrier leading to neurological effects.<sup>14</sup>

Some of the effects of PTX are due to the binding of the toxin to the cell, not to the enzymatic inactivation of G proteins. These effects are often shown to occur with PTX, or with either toxoid or genetically engineered toxin with an altered enzyme activity. Studies indicate that mutant pertussis toxin possesses adjuvant properties with the ability to encourage both local and systemic responses, to promote T helper cell responses to co-administered antigens and to favor the production of Th1/Th17 cells, important in mediating host immunity to infectious pathogens.<sup>15</sup> PTX binds to the cell receptor, TLR4 which activates Rac and subsequently causes various effects depending on the type of cell treated.<sup>4</sup> The toxin or binding oligomer induces dendritic cell maturation in a TLR4-dependent manner.<sup>16</sup> Effects of PTX intoxication, both dependent and independent of G proteins are reviewed by Locht *et al.*<sup>7</sup> and Mangmool *et al.*<sup>17</sup>

Pertussis toxin has been variously referred to in literature as lymphocytosis-promoting factor, islet-activating protein, histamine-sensitizing factor, hemagglutinin and pertussigen. The toxin is useful in creating animal models of autoimmune disease. For example, pertussis toxin is administered to mice along with myelin antigen to create a mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE).<sup>18,19</sup>

Pertussis toxin from List Biological Laboratories is isolated from *Bordetella pertussis* strain 165. This preparation is highly purified and contains all five subunits. It is tested to assure minimal adenylate cyclase activity in the presence of calmodulin.<sup>20,21</sup> Each lot is tested for activity in the CHO cell assay as described by Hewlett *et al.*<sup>22</sup>

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List Labs produces Pertussis Toxin Mutant R9K, E129A, a genetically inactivated mutant of pertussis toxin, which has a modified sequence encoding the enzyme subunit. Virulence of this pertussis mutant is reduced relative to that found with the wild type.<sup>23</sup> The pertussis mutant protein is isolated from the TY-178 strain of *Bordetella bronchiseptica* which contains a genetically modified sequence encoding the S1 subunit. Genetically inactivated toxin may be used as an antigen or as a carrier to promote an immune response.

Native pertussis toxin is supplied in three formulations (List Labs Prod. #179, 180 and 181), in glycerol, lyophilized, and lyophilized salt-free. Pertussis Toxin Mutant (Prod. #184) is supplied lyophilized. A detailed lot analysis documenting purity and biological activity plus complete instructions on reconstitution and storage accompany each shipment.

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

### Ordering Information

Product No.	Description	Size
<a href="#">179A,B</a>	Pertussis Toxin, in Glycerol	50 µg, 200 µg
<a href="#">180</a>	Pertussis Toxin, Lyophilized in Buffer	50 µg
<a href="#">181</a>	Pertussis Toxin, Lyophilized, Salt-Free	50 µg
<a href="#">184</a>	Pertussis Toxin Mutant	50 µg

### Related Products

Filamentous Hemagglutinin (FHA) from *B. pertussis*, Product [#170](#)  
Fimbriae 2/3 from *B. pertussis*, Product [#186](#)  
Pertactin from *B. pertussis* (69 kDa Protein), Product [#187](#)  
Adenylate Cyclase Toxin, Recombinant from *B. pertussis*, Frozen Liquid, Product [#188L](#)  
Adenylate Cyclase, Recombinant, Reduced Endotoxin, Frozen Liquid, Product [#197L](#)  
Adenylate Cyclase Toxoid, Recombinant, Frozen Liquid, Product [#198L](#)  
HPT™ LPS, highly purified from *B. pertussis* 165, Product [#400](#)

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