ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Recombinant Protective Antigen 20 (PA20) Protein (*B. Anthracis*)

□ Cat. # PA20-R PA20 Purified, recombinant Protein **SIZE**: 50 ug

After inhalation by mammals, *Bacillus anthracis* spores germinate in alveolar macrophages then migrate to lymph nodes where they multiply. The vegetative bacteria excrete the tripartite exotoxin which consists of three polypeptides: protective antigen (**PA**, 83 kDa), lethal factor (LF, 90 kDa) and oedma factor (OF, 89 kDa). The two components (OF and LF) of the toxin enzymatically modify substrates within the cytosol of the mammalian cells: The **OF** is an adenylate cyclase that impairs the host defenses through a variety of mechanisms inhibiting phagocytosis. The **LF** is a zinc dependent protease that cleaves several mitogen activated protein kinase kinases **(MAPKK)** and causes lysis of macrophages. To intoxicate mammalian cells, the third component of the toxin **PA,** binds to a ubiquitously expressed cellular receptor, Tumor Endothelium Marker-8 (**TEM8**). Upon binding to TEM8, PA is cleaved into 20 and 63kDa fragments (PA20 and PA63) by furin or furin-like proteases. PA20 dissociates into medium and allows the PA63 fragment to heptamerize and bind LF and OF of the toxin. The resulting complex of **PA63** fragment with EF and/or OF binds to PA-receptor TEM8/ATR and internalized into endosomes followed by translocation of LF and OF into cytosol of the cells.

B. anthracis PA83 is the proteolytically activated in vivo by a furin-like protease to produce a 20 kDa fragment (PA20) from the N-terminus. The remaining 63 kDa portion, PA63, may oligomerize into a ring-shaped heptamer. Although the C-terminal region of both PA63 and PA83 is capable of binding to the cell receptor, cleavage of PA is an essential step in exposing the binding sites for EF and LF. Cleavage also allows the formation of the heptamer. Each heptamer attached to the surface of a cell has the ability to bind up to three molecules of LF and/or EF. The complex formed between PA heptamer and EF or LF is taken into the cell by receptor mediated endocytosis. Following endocytosis, the acidified environment within the endosome triggers the heptamer to act as a pore releasing LF or EF into the cytosol where they attack their targets. PA is one of the three protein components of anthrax toxin, protective antigen (PA) is the central moiety that mediates the entry of lethal factor and edema factor into the target cell. PA binds to the cell surface via a type I membrane protein with a von Willebrand factor A domain called anthrax toxin receptor.

Source of Protein

PA20 (~20 Kda) is produced from purified PA83 by trypsin cleavage and purified (>95-98%). Some smaller bands may also appear particularly after prolong storage. It is provided as 50ug/100ul in 5mM Hepes, 50 mM NaCl, pH 7.5 buffer in liquid or lyophilized in this buffer (50 ug or other sizes). The product is not necessarily sterile. The lyophilized products should be reconstituted with 100 ul of distilled water and filter through 0.2 u if sterility is desired. It may help to add 1% BSA or HSA (albumin) to maintain PA protein activity. It can then be used or aliquoted for storage in small aliquots at -70oC or below.

Recommended Usage

May be used for ELISA or Western as positive control. Not intended for in vivo use.

For cell culture applications, medium containing glutamine must be fresh. Ammonium ion released when glutamine breaks down may prevent acidification of the endosome thereby inhibiting translocation of LF or EF into the cytosol4. A stable form of glutamine may be used.

General References

Bradley KA et al (2001) Nature 414, 225-229; liu S and Leppla SH (2002) JBC (in press); Leppla, SH (1982) PNAS 79, 3182; O'Brien J et al (1985) Infect Immun 47, 306; Duesbery, NS et al (1998) Science 280, 734

Storage

Short-term: unopened, undiluted vials for less than a week at 4oC.

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.

Shipping: 4oC for solutions and room temp for powder.

MSDS: Anthrax proteins (LF, PA, and EF) are produced by plasmids from a non-sporulating avirulent strain of Bacillus anthracis which lacks both of the wild type plasmids, pX01 and pX02. This host makes none of the anthrax toxin components and no polygutamate capsule. Thus, the anthrax toxin components produced are single, pure proteins lacking all other virulence factors. Individually, each protein is non-toxic and presents no hazard during normal laboratory use. However, normal GLP procedures should be observed when handling this product and all areas cleaned after usage.

**This product is for In vitro research use only.*

Related materials available from ADI

Antibodies: ATR11-A, ATR12-A, ATR31-A

PA20-R 130801A

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