

Product Specification Sheet

Anti- B. Anthracis Protective Antigen (PA) Antibodies

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| Cat. # PA17-A | Polyclonal Rabbit Anti-Protective antigen 83 (PA83) peptide IgG | SIZE: 100 ug |
| Cat. # PA17-C | Recombinant purified protective antigen 83 (PA83) protein control for WB | SIZE: 100 ul |

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.

Source of Antigen and Antibodies

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|---------------------|---|
| Antigen | synthetic peptide corresponding to 14 amino acids at the CT of the Anthrax protective antigen protein (accession #AAF86457).(1) |
| Ab Host/type | Rabbit polyclonal IgG, purified IgG supplied in PBS, pH 7.4, 0.1% BSA, 0.05% azide |
| 2-ab | Goat Anti-rabbit IgG-HRP cat # 20320 (AP, biotin, FITC conjugates also available) |
| -ve control | # 20009-1, Rabbit (non-immune) IgG, purified, suitable for ELISA, Western, IHC as -ve control |

Cat # PA17-C

B. Anthracis protective antigen 83 (PA83) was expressed in E. coli and purified. Single major band by SDS-PAGE, Mol wt 83Kda (purity >90-95%). A smaller band, a proteolytic form of PA83, PA63 or PA20 may also appear. For Western blot +ve control (Cat # PA17-C) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of PA17-C for good visibility with polyclonal rabbit antibody Cat # PA17-A or mouse monoclonal #PA11-M. Store at -20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the PA17-C solution prior to heating and loading on gels. This preparation is not

biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

Form & Storage of Antibodies/Peptide Control

Pure IgG (Cat # PA17-A)

100 ug/100ul solution lyophilized powder
Supplied in: PBS, pH 7.4, 0.1% BSA, 0.05% azide
Reconstitute powder in PBS at 1 mg/ml

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.

Recommended Usage

Western Blotting (1-10 ug/ml for affinity pure antibody using ECL technique).

ELISA: Control proteins can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (0.1-1 ug/ml). We recommend the following pairs for sandwich assay:

Histochemistry & Immunofluorescence: Not tested.

Specificity & Cross-reactivity

Rabbit polyclonal antibody (cat # PA17-A) do not cross-react with Lethal Factor (LF) of *Bacillus anthracis*, *Y. Pestis*, *F. Tularensis* and *Toxoplasma gondi*. The cross-reactivity in various species is not known. Since the epitope of this antibody is located near the C-terminus, the antibody will recognize both PA63 and PA83 but not PA20 fragment. Recombinant purified proteins PA83 (Cat # PA83-C) and PA63 (Cat #PA63-C) can be used a positive controls.

General References

(1) 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

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

Related materials available from ADI

Antibodies: ATR11-A, ATR12-A, ATR31-A
Recombinant PA20, PA63, PA83 proteins and ELISA kits
ELISA kits for the detection of anti-PA, LF, EF in mouse, rabbit, and goat immunized with various anthrax proteins
ELISA kit for measurement of PA83
PA17-A-C 80125A