

## Product Specification Sheet

### Protective Antigen 63 (PA63) Protein control

□ Cat. # PA63-C

PA63 Purified, recombinant Protein control for Western Blot

**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 oedema 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 63kD protein PA63. It 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

Recombinant purified PA63 protein (>95% pure, 63 kda major band) for Western blot +ve control (**Cat # PA63-C**) is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **PA63-C** for good visibility with an appropriate antibody. 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 **PA63-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.

#### **Recommended Usage**

Western as positive control. .

#### **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.

*\*This product is for In vitro research use only. It must not be resold, Re-packaged, or sold to 3<sup>rd</sup> parties without written approval from ADI.*

#### **Related materials available from ADI**

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

PA63-C

80715A