Anthrax Protective Antigen (PA), Recombinant from *Bacillus anthracis*

Catalog No. NR-140

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Contributor:

NIH - AIDS Research and Reference Reagent Program.

Manufacturer:

List Biological Laboratories, Inc.

Product Description:

Recombinant anthrax protective antigen (PA, 83 kDa) was produced using a plasmid licensed from the NIH. The plasmid was introduced into a non-sporulating avirulent strain of *Bacillus anthracis* lacking both of the wild type plasmids, pX01 and pX02. Recombinant PA was purified using conventional chromatographic techniques. The resulting purified protein lacks all other anthrax virulence factors.

In vivo, recombinant PA binds to surface receptors on the mammalian cell membrane, and is cleaved by a cellular protease to a 63 kDa protein. When combined with recombinant lethal factor (LF) or edema factor (EF), the cleaved PA binds the toxin enzyme component and mediates its transportation into the cytosol where it exerts its pathogenic effect.

Material Provided:

Each vial contains approximately 0.93 mg of recombinant PA. When reconstituted with 1 mL of sterile distilled water, the concentration of buffer is 5 mM HEPES (pH 7.5) and 50 mM NaCl.

Packaging and Storage:

This product was packaged aseptically, lyophilized and sealed under vacuum. The product is provided at room temperature and should be stored at 2°C to 8°C prior to reconstitution.

Reconstitution and Storage:

Recombinant anthrax PA reconstituted in sterile distilled water is stable for a few hours at 4°C. Longer periods of time at 4°C will result in a decline in the activity of PA-LF complex in living cells.

To enhance stability and recovery, reconstitution at 1 mg/mL[1] in the presence of 1 mg/mL bovine serum albumin (BSA) is recommended. Under these conditions, storage for a period of two weeks at 4°C may be acceptable for some applications.

For optimal long-term storage, aliquoting and freezing the material at -20°C or -80°C is recommended. Repeated freeze-thaw cycles should be avoided. Glycerol may be added to 50% if a liquid is desired at freezer temperatures.

Concentration:

Protein concentration was determined by a modification of the method of Bradford,[2] using BSA as the standard.

Activation:

In certain systems, this product may require trypsinization to generate the active C-terminal 63 kDa fragment.[3]

Tissue Culture Application:

Tissue culture media containing glutamate must be fresh. Ammonium ion released when glutamate breaks down may prevent acidification of the endosome thereby inhibiting translocation of LF or EF into the cytosol.[4] A stable form of glutamate may be used.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Anthrax Protective Antigen (PA), Recombinant from *Bacillus anthracis*, NR-140."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Leppla, S. H. "Production and Purification of Anthrax Toxin." Methods Enzymol. 165 (1988): 103–116. PubMed: 3148094.
2. Bradford, M. M. "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding." Anal. Biochem. 72 (1976): 248–254. PubMed: 942051.
3. Bhatnagar, R., Y. Singh, S. H. Leppla, A. M. Friedlander. "Calcium is Required for the Expression of Anthrax Lethal Toxin Activity in the Macrophage-like Cell Line J774A.1." Infect. Immun. 57(7) (1989): 2107–2114. PubMed: 2499545.
4. Stephen Little, personal communication.

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