Product Information Sheet for NR-36210

Anthrax Edema Factor (EF), Recombinant from *Bacillus anthracis*

Catalog No. NR-36210

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For research use only. Not for human use.

Contributor:

BEI Resources

Manufacturer:

List Biological Laboratories, Inc.

Product Description:

Recombinant anthrax edema factor (EF, 89 kDa) was produced using a plasmid licensed from the NIH.[1,2] The plasmid was introduced into a non-sporulating avirulent strain of *Bacillus anthracis* lacking both of the wild type plasmids, pX01 and pX02. Recombinant EF was purified using conventional chromatographic techniques. The resulting purified protein lacks all other anthrax virulence factors.

There is one predicted amino acid difference between NR-36210 and NR-141. NR-141 contains an asparagine, rather than a serine, at amino acid position 415 (amino acid position 444 of the precursor protein for NR-141, amino acid position 447 of precursor EF, GenPept P40136, amino acid position 414 of mature EF, GenPept P40136.

There is one expected amino acid difference between NR-36210 and mature EF, GenPept P40136. NR-36210 may have an additional histidine residue at the N-terminus (amino acid position 30 of the precursor protein for NR-36210). This has not yet been confirmed by N-terminal sequencing for NR-36210.

EF is a calmodulin-dependent adenylate cyclase, and its enzymatic activity results in an increase in intracellular cAMP levels. In addition, EF inhibits the immune response by removing calmodulin from involvement in calcium-triggered signaling. *In vivo*, recombinant EF binds to a cleaved form of recombinant protective antigen (PA), and is transported by cleaved PA into the cytosol of the mammalian cell, where EF exerts its pathogenic effect.

Material Provided:

Each vial contains 100 µg of recombinant EF from *Bacillus anthracis*. When reconstituted with 0.1 mL of sterile distilled water, the concentration of buffer is 5 mM HEPES (pH 7.5) and 50 mM NaCl. Note: Handle the product gently; DO NOT VORTEX.

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 EF reconstituted in sterile distilled water is stable for a few hours at 2°C to 8°C. Longer periods of time at 2°C to 8°C will result in a decline in the enzymatic activity of EF.

To enhance stability and recovery, reconstitution at 1 mg/mL in the presence of 1 mg/mL bovine serum albumin (BSA) is recommended. Under these conditions, storage for a period of two weeks at 2°C to 8°C may be acceptable for some applications.

For optimal long-term storage, aliquoting and freezing the material at -20°C or colder 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,[4] using BSA as the standard.

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 lethal factor (LF) or EF into the cytosol.[5] 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 Edema Factor (EF), Recombinant from *Bacillus anthracis*, NR-36210."

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.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

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References:

1. Leppla, S. H. "Production and Purification of Anthrax Toxin." Methods Enzymol. 165 (1988): 103–116. PubMed: 3148094.
2. Leppla, S. H. "Purification and Characterization of Adenylyl Cyclase from *Bacillus anthracis*." Methods Enzymol. 195 (1991): 153–168. PubMed: 1903483.
3. Escuyer, V., et al. "Structural Homology between Virulence-Associated Bacterial Adenylate Cyclases." Gene 71 (1988): 293–298. PubMed: 2906312. GenPept: P40136.
4. 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.
5. Stephen Little, personal communication.

Support Provided by NIAID

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