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

Catalog No. NR-2585

This reagent is the property of the U.S. Government.

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.

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.

NR-2585 demonstrated comparable adenylate cyclase activity to BEI Resources NR-141 in side-by-side experiments in October, 2005.

The predicted protein sequences of precursor EF protein, GenPept P40136[3], and the precursor proteins for NR-2585, BEI Resources NR-2587, and NR-141 are compared in Table 1. The predicted protein sequence of NR-2585 is highlighted. The signal peptides are underlined.

Note: There is one expected amino acid difference between NR-2585 and mature EF, GenPept P40136. NR-2585 may have an additional histidine residue at the N-terminus (shown in bold in Table 1; amino acid position 30 of the precursor protein for NR-2585). This additional histidine residue has not yet been confirmed by N-terminal sequencing for NR-2585; however, it has been confirmed for NR-141 (shown in bold in Table 1). There is one predicted amino acid difference between NR-2585 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; shown in bold and identified by asterisks in Table 1).

Material Provided:

Each vial contains 177 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), 50 mM NaCl and 5 mM EDTA.

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 4°C. Longer periods of time at 4°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 4°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-2585."

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.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale. This material may be subject to third party patent rights.

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.

ATCC® is a trademark of the American Type Culture Collection.

Support Provided by NIAID

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Table 1 – Predicted Precursor EF Protein Sequences (Signal Peptides Underlined)

```
                1                                                   50
 GenPept P40136 MTRNKFIPNK FSIISFSVLL FAISSSQAIE VNAMNEHYTE SDIKRNHKTE
NR-2585/NR-2587    MKKRKVL IPLMALSTIL VSSTGNLEVI QAHMNEHYTE SDIKRNHKTE
         NR-141    MKKRKVL IPLMALSTIL VSSTGNLEVI QAHMNEHYTE SDIKRNHKTE
                   1                                                   47

                51                                                  100
 GenPept P40136 KNKTEKEKFK DSINNLVKTE FTNETLDKIQ QTQDLLKKIP KDVLEIYSEL
NR-2585/NR-2587 KNKTEKEKFK DSINNLVKTE FTNETLDKIQ QTQDLLKKIP KDVLEIYSEL
         NR-141 KNKTEKEKFK DSINNLVKTE FTNETLDKIQ QTQDLLKKIP KDVLEIYSEL
                48                                                  97

               101                                                  150
 GenPept P40136 GGEIYFTDID LVEHKELQDL SEEEKNSMNS RGEKVPFASR FVFEKKRETP
NR-2585/NR-2587 GGEIYFTDID LVEHKELQDL SEEEKNSMNS RGEKVPFASR FVFEKKRETP
         NR-141 GGEIYFTDID LVEHKELQDL SEEEKNSMNS RGEKVPFASR FVFEKKRETP
                98                                                  147

               151                                                  200
 GenPept P40136 KLIINIKDYA INSEQSKEVY YEIGKGISLD IISKDKSLDP EFLNLIKSLS
NR-2585/NR-2587 KLIINIKDYA INSEQSKEVY YEIGKGISLD IISKDKSLDP EFLNLIKSLS
         NR-141 KLIINIKDYA INSEQSKEVY YEIGKGISLD IISKDKSLDP EFLNLIKSLS
                148                                                 197

               201                                                  250
 GenPept P40136 DDSDSSDLLF SQKFKEKLEL NNKSIDINFI KENLTEFQHA FSLAFSYYFA
NR-2585/NR-2587 DDSDSSDLLF SQKFKEKLEL NNKSIDINFI KENLTEFQHA FSLAFSYYFA
         NR-141 DDSDSSDLLF SQKFKEKLEL NNKSIDINFI KENLTEFQHA FSLAFSYYFA
                198                                                 247

               251                                                  300
 GenPept P40136 PDHRTVLELY APDMFEYMNK LEKGGFEKIS ESLKKEGVEK DRIDVLKGEK
NR-2585/NR-2587 PDHRTVLELY APDMFEYMNK LEKGGFEKIS ESLKKEGVEK DRIDVLKGEK
         NR-141 PDHRTVLELY APDMFEYMNK LEKGGFEKIS ESLKKEGVEK DRIDVLKGEK
                248                                                 297

               301                                                  350
 GenPept P40136 ALKASGLVPE HADAFKKIAR ELNTYILFRP VNKLATNLIK SGVATKGLNV
NR-2585/NR-2587 ALKASGLVPE HADAFKKIAR ELNTYILFRP VNKLATNLIK SGVATKGLNV
         NR-141 ALKASGLVPE HADAFKKIAR ELNTYILFRP VNKLATNLIK SGVATKGLNV
                298                                                 347

               351                                                  400
 GenPept P40136 HGKSSDWGPV AGYIPFDQDL SKKHGQQLAV EKGNLENKKS ITEHEGEIGK
NR-2585/NR-2587 HGKSSDWGPV AGYIPFDQDL SKKHGQQLAV EKGNLENKKS ITEHEGEIGK
         NR-141 HGKSSDWGPV AGYIPFDQDL SKKHGQQLAV EKGNLENKKS ITEHEGEIGK
                348                                                 397

               401                                                 *450
 GenPept P40136 IPLKLDHLRI EELKENGIIL KGKKEIDNGK KYYLLESNNQ VYEFRISDEN
NR-2585/NR-2587 IPLKLDHLRI EELKENGIIL KGKKEIDNGK KYYLLESNNQ VYEFRISDEN
         NR-141 IPLKLDHLRI EELKENGIIL KGKKEIDNGK KYYLLESNNQ VYEFRINDEN
                398                                                 *447

               451                                                  500
 GenPept P40136 NEVQYKTKEG KITVLGEKFN WRNIEVMAKN VEGVLKPLTA DYDLFALAPS
NR-2585/NR-2587 NEVQYKTKEG KITVLGEKFN WRNIEVMAKN VEGVLKPLTA DYDLFALAPS
         NR-141 NEVQYKTKEG KITVLGEKFN WRNIEVMAKN VEGVLKPLTA DYDLFALAPS
                448                                                 497

               501                                                  550
 GenPept P40136 LTEIKKQIPQ KEWDKVVNTP NSLEKQKGVT NLLIKYGIER KPDSTKGTLS
NR-2585/NR-2587 LTEIKKQIPQ KEWDKVVNTP NSLEKQKGVT NLLIKYGIER KPDSTKGTLS
         NR-141 LTEIKKQIPQ KEWDKVVNTP NSLEKQKGVT NLLIKYGIER KPDSTKGTLS
                498                                                 547
```

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

```
                551                                                 600
 GenPept P40136 NWQKQMLDRL NEAVKYTGYT GGDVVNHGTE QDNEEFPEKD NEIFIINPEG
NR-2585/NR-2587 NWQKQMLDRL NEAVKYTGYT GGDVVNHGTE QDNEEFPEKD NEIFIINPEG
         NR-141 NWQKQMLDRL NEAVKYTGYT GGDVVNHGTE QDNEEFPEKD NEIFIINPEG
                548                                                 597

                601                                                 650
 GenPept P40136 EFILTKNWEM TGRFIEKNIT GKDYLYYFNR SYNKIAPGNK AYIEWTDPIT
NR-2585/NR-2587 EFILTKNWEM TGRFIEKNIT GKDYLYYFNR SYNKIAPGNK AYIEWTDPIT
         NR-141 EFILTKNWEM TGRFIEKNIT GKDYLYYFNR SYNKIAPGNK AYIEWTDPIT
                598                                                 647

                651                                                 700
 GenPept P40136 KAKINTIPTS AEFIKNLSSI RRSSNVGVYK DSGDKDEFAK KESVKKIAGY
NR-2585/NR-2587 KAKINTIPTS AEFIKNLSSI RRSSNVGVYK DSGDKDEFAK KESVKKIAGY
         NR-141 KAKINTIPTS AEFIKNLSSI RRSSNVGVYK DSGDKDEFAK KESVKKIAGY
                648                                                 697

                701                                                 750
 GenPept P40136 LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSKQ IAPEYKNYFQ
NR-2585/NR-2587 LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSKQ IAPEYKNYFQ
         NR-141 LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSKQ IAPEYKNYFQ
                698                                                 747

                751                                                 800
 GenPept P40136 YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK
NR-2585/NR-2587 YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK
         NR-141 YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK
                748                                                 797
```