

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

**Catalog No. NR-2587**

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.<sup>1,2</sup> 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-2587 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<sup>3</sup>, and the precursor proteins for BEI Resources NR-2585, NR-2587, and NR-141 are compared in Table 1. The predicted protein sequence of NR-2587 is highlighted. The signal peptides are underlined.

**Note:** There is one expected amino acid difference between NR-2587 and mature EF, GenPept P40136. NR-2587 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-2587). This additional histidine residue has not yet been confirmed by N-terminal sequencing for NR-2587; however, it has been confirmed for NR-141 (shown in bold in Table 1). There is one predicted amino acid difference between NR-2587 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 884 µg of recombinant EF from *Bacillus anthracis*. When reconstituted with 0.5 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,<sup>4</sup> 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.<sup>5</sup> 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-2587."

**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](http://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.

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](http://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.



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

		1		50
GenPept	P40136	<u>MTRNKFIPNK</u>	<u>FSIISFSVLL</u>	<u>FAISSSQAI</u>
NR-2585/NR-2587		<u>MKKRKVL</u>	<u>IPLMALSTIL</u>	<u>VSSTGNLEVI</u>
NR-141		<u>MKKRKVL</u>	<u>IPLMALSTIL</u>	<u>VSSTGNLEVI</u>
		1		47
		51		100
GenPept	P40136	<u>KNKTEKEKFK</u>	<u>DSINNLVKTE</u>	<u>FTNETLDKIQ</u>
NR-2585/NR-2587		<u>KNKTEKEKFK</u>	<u>DSINNLVKTE</u>	<u>FTNETLDKIQ</u>
NR-141		<u>KNKTEKEKFK</u>	<u>DSINNLVKTE</u>	<u>FTNETLDKIQ</u>
		48		97
		101		150
GenPept	P40136	<u>GGEIYFTDID</u>	<u>LVEHKELQDL</u>	<u>SEEEKNSMNS</u>
NR-2585/NR-2587		<u>GGEIYFTDID</u>	<u>LVEHKELQDL</u>	<u>SEEEKNSMNS</u>
NR-141		<u>GGEIYFTDID</u>	<u>LVEHKELQDL</u>	<u>SEEEKNSMNS</u>
		98		147
		151		200
GenPept	P40136	<u>KLIINIKDYA</u>	<u>INSEQSKEVY</u>	<u>YEIGKGISLD</u>
NR-2585/NR-2587		<u>KLIINIKDYA</u>	<u>INSEQSKEVY</u>	<u>YEIGKGISLD</u>
NR-141		<u>KLIINIKDYA</u>	<u>INSEQSKEVY</u>	<u>YEIGKGISLD</u>
		148		197
		201		250
GenPept	P40136	<u>DDSDSSDLLF</u>	<u>SQKFKEKLEL</u>	<u>NNKSIDINFI</u>
NR-2585/NR-2587		<u>DDSDSSDLLF</u>	<u>SQKFKEKLEL</u>	<u>NNKSIDINFI</u>
NR-141		<u>DDSDSSDLLF</u>	<u>SQKFKEKLEL</u>	<u>NNKSIDINFI</u>
		198		247
		251		300
GenPept	P40136	<u>PDHRTVLELY</u>	<u>APDMFEYMNK</u>	<u>LEKGGFEKIS</u>
NR-2585/NR-2587		<u>PDHRTVLELY</u>	<u>APDMFEYMNK</u>	<u>LEKGGFEKIS</u>
NR-141		<u>PDHRTVLELY</u>	<u>APDMFEYMNK</u>	<u>LEKGGFEKIS</u>
		248		297
		301		350
GenPept	P40136	<u>ALKASGLVPE</u>	<u>HADAFKKIAR</u>	<u>ELNTYILFRP</u>
NR-2585/NR-2587		<u>ALKASGLVPE</u>	<u>HADAFKKIAR</u>	<u>ELNTYILFRP</u>
NR-141		<u>ALKASGLVPE</u>	<u>HADAFKKIAR</u>	<u>ELNTYILFRP</u>
		298		347
		351		400
GenPept	P40136	<u>HGKSSDWGPV</u>	<u>AGYIPFDQDL</u>	<u>SKKHGQQLAV</u>
NR-2585/NR-2587		<u>HGKSSDWGPV</u>	<u>AGYIPFDQDL</u>	<u>SKKHGQQLAV</u>
NR-141		<u>HGKSSDWGPV</u>	<u>AGYIPFDQDL</u>	<u>SKKHGQQLAV</u>
		348		397
		401		*450
GenPept	P40136	<u>IPLKLDHLRI</u>	<u>EELKENGIL</u>	<u>KGKKEIDNGK</u>
NR-2585/NR-2587		<u>IPLKLDHLRI</u>	<u>EELKENGIL</u>	<u>KGKKEIDNGK</u>
NR-141		<u>IPLKLDHLRI</u>	<u>EELKENGIL</u>	<u>KGKKEIDNGK</u>
		398		*447
		451		500
GenPept	P40136	<u>NEVQYKTEG</u>	<u>KITVLGEKFN</u>	<u>WRNIEVMAKN</u>
NR-2585/NR-2587		<u>NEVQYKTEG</u>	<u>KITVLGEKFN</u>	<u>WRNIEVMAKN</u>
NR-141		<u>NEVQYKTEG</u>	<u>KITVLGEKFN</u>	<u>WRNIEVMAKN</u>
		448		497
		501		550
GenPept	P40136	<u>LTEIKKQIPQ</u>	<u>KEWDKVVNTP</u>	<u>NSLEKQKGV</u>
NR-2585/NR-2587		<u>LTEIKKQIPQ</u>	<u>KEWDKVVNTP</u>	<u>NSLEKQKGV</u>
NR-141		<u>LTEIKKQIPQ</u>	<u>KEWDKVVNTP</u>	<u>NSLEKQKGV</u>
		498		547

	551		600
GenPept P40136	NWQKQMLDRL NEAVKYTGYT GGDVVNHGTE QDNEEFPEKD NEIFIINPEG		
NR-2585/NR-2587	<b>NWQKQMLDRL NEAVKYTGYT GGDVVNHGTE QDNEEFPEKD NEIFIINPEG</b>		
NR-141	NWQKQMLDRL NEAVKYTGYT GGDVVNHGTE QDNEEFPEKD NEIFIINPEG		
	548		597
	601		650
GenPept P40136	EFILTKNWEM TGRFIEKNIT GKDYLYYFNR SYNKIAPGNK AYIEWTDPIT		
NR-2585/NR-2587	<b>EFILTKNWEM TGRFIEKNIT GKDYLYYFNR SYNKIAPGNK AYIEWTDPIT</b>		
NR-141	EFILTKNWEM TGRFIEKNIT GKDYLYYFNR SYNKIAPGNK AYIEWTDPIT		
	598		647
	651		700
GenPept P40136	KAKINTIPTS AEFIKNLSSI RRSSNVGVYK DSGDKDEFAK KESVKKIAGY		
NR-2585/NR-2587	<b>KAKINTIPTS AEFIKNLSSI RRSSNVGVYK DSGDKDEFAK KESVKKIAGY</b>		
NR-141	KAKINTIPTS AEFIKNLSSI RRSSNVGVYK DSGDKDEFAK KESVKKIAGY		
	648		697
	701		750
GenPept P40136	LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSQ IAPEYKNYFQ		
NR-2585/NR-2587	<b>LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSQ IAPEYKNYFQ</b>		
NR-141	LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSQ IAPEYKNYFQ		
	698		747
	751		800
GenPept P40136	YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK		
NR-2585/NR-2587	<b>YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK</b>		
NR-141	YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK		
	748		797