

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

Catalog No. NR-141

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Contributor:

NIH - AIDS Research and Reference Reagent Program

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-141 demonstrated comparable adenylate cyclase activity to BEI Resources NR-2585 and NR-2587 in side-by-side experiments in October, 2005.

The predicted protein sequences of precursor EF protein, GenPept P40136³, and the precursor proteins for NR-2585, NR-2587, and NR-141 are compared in Table 1. The predicted protein sequence of NR-141 is highlighted. The signal peptides are underlined.

Note: There are two predicted amino acid differences between NR-141 and mature EF, GenPept P40136. 1) NR-141 has an additional histidine residue at the N-terminus (shown in bold in Table 1; amino acid position 30 of the precursor protein for NR-141). This additional histidine residue has been confirmed by N-terminal sequencing of NR-141. 2) 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 approximately 0.32 mg 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) 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 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-141."

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/bmb15/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. 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.

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

GenBank P40136 NR-2585/NR-2587 NR-141	1	MTRNKFIPNK <u>FSIISFSVLL</u> FAISSSQAI E VNAMEHYTE SDIKRNHKTE	50
		<u>MKKRKVL IPLMALSTIL VSSTGNLEVI QA</u> HMNEHYTE SDIKRNHKTE	
		<u>MKKRKVL IPLMALSTIL VSSTGNLEVI QA</u> HMNEHYTE <u>SDIKRNHKTE</u>	47
	1		47
GenBank P40136 NR-2585/NR-2587 NR-141	51	KNKTEKEKFK DSINNLVKTE FTNETLDKIQ QTQDLLKKIP KDVLEIYSEL	100
		KNKTEKEKFK DSINNLVKTE FTNETLDKIQ QTQDLLKKIP KDVLEIYSEL	
		<u>KNKTEKEKFK DSINNLVKTE FTNETLDKIQ QTQDLLKKIP KDVLEIYSEL</u>	97
	48		97
GenBank P40136 NR-2585/NR-2587 NR-141	101	GGEIYFTDID LVEHKELQDL S EEEKNSMNS RGEKVPFASR FVFEKKRETP	150
		GGEIYFTDID LVEHKELQDL S EEEKNSMNS RGEKVPFASR FVFEKKRETP	
		<u>GGEIYFTDID LVEHKELQDL S EEEKNSMNS RGEKVPFASR FVFEKKRETP</u>	147
	98		147
GenBank P40136 NR-2585/NR-2587 NR-141	151	KLIINIKDYA INSEQSKEYV YEIGKGISLD IISKDKSLDP EFLNLIKSL S	200
		KLIINIKDYA INSEQSKEYV YEIGKGISLD IISKDKSLDP EFLNLIKSL S	
		<u>KLIINIKDYA INSEQSKEYV YEIGKGISLD IISKDKSLDP EFLNLIKSL S</u>	197
	148		197
GenBank P40136 NR-2585/NR-2587 NR-141	201	DDSDSSDLLF SQKFKEKLEL NNKSIDINFI KENLTEFQHA FSLAFSYYFA	250
		DDSDSSDLLF SQKFKEKLEL NNKSIDINFI KENLTEFQHA FSLAFSYYFA	
		<u>DDSDSSDLLF SQKFKEKLEL NNKSIDINFI KENLTEFQHA FSLAFSYYFA</u>	247
	198		247
GenBank P40136 NR-2585/NR-2587 NR-141	251	PDHRTVLELY APDMFEYMNK LEKGGFEKIS ESLKKEGVEK DRIDVLKGEK	300
		PDHRTVLELY APDMFEYMNK LEKGGFEKIS ESLKKEGVEK DRIDVLKGEK	
		<u>PDHRTVLELY APDMFEYMNK LEKGGFEKIS ESLKKEGVEK DRIDVLKGEK</u>	297
	248		297
GenBank P40136 NR-2585/NR-2587 NR-141	301	ALKASGLVPE HADAFKKIAR ELNTYILFRP VNKLATNLIK SGVATKGLNV	350
		ALKASGLVPE HADAFKKIAR ELNTYILFRP VNKLATNLIK SGVATKGLNV	
		<u>ALKASGLVPE HADAFKKIAR ELNTYILFRP VNKLATNLIK SGVATKGLNV</u>	347
	298		347
GenBank P40136 NR-2585/NR-2587 NR-141	351	HGKSSDWGPV AGYIPFDQDL SKKHGQQLAV EKG NLENKKS ITEHEGEIGK	400
		HGKSSDWGPV AGYIPFDQDL SKKHGQQLAV EKG NLENKKS ITEHEGEIGK	
		<u>HGKSSDWGPV AGYIPFDQDL SKKHGQQLAV EKG NLENKKS ITEHEGEIGK</u>	397
	348		397
GenBank P40136 NR-2585/NR-2587 NR-141	401	IPLKLDHLRI EELKENG IIL KGKKEIDNGK KYLLESNNQ VYEFRI SDEN	*450
		IPLKLDHLRI EELKENG IIL KGKKEIDNGK KYLLESNNQ VYEFRI SDEN	
		<u>IPLKLDHLRI EELKENG IIL KGKKEIDNGK KYLLESNNQ VYEFRI SDEN</u>	*447
	398		*447
GenBank P40136 NR-2585/NR-2587 NR-141	451	NEVQYKTEG KITVLGEKFN WRNIEVMAKN VEGVLKPLTA DYDLFALAPS	500
		NEVQYKTEG KITVLGEKFN WRNIEVMAKN VEGVLKPLTA DYDLFALAPS	
		<u>NEVQYKTEG KITVLGEKFN WRNIEVMAKN VEGVLKPLTA DYDLFALAPS</u>	497
	448		497
GenBank P40136 NR-2585/NR-2587 NR-141	501	LTEIKKQIPQ KEWDKVVNTP NSLEKQKGV T NLLIKYGI ER KPDSTKGTLS	550
		LTEIKKQIPQ KEWDKVVNTP NSLEKQKGV T NLLIKYGI ER KPDSTKGTLS	
		<u>LTEIKKQIPQ KEWDKVVNTP NSLEKQKGV T NLLIKYGI ER KPDSTKGTLS</u>	
	498		

	551		600
GenBank 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
GenBank 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
GenBank 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
GenBank P40136	LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSQ IAPEYKNYFQ		
NR-2585/NR-2587	LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSQ IAPEYKNYFQ		
NR-141	LSDYYNSANH IFSQEKKRKI SIFRGIQAYN EIENVLKSQ IAPEYKNYFQ		
	698		747
	751		800
GenBank P40136	YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK		
NR-2585/NR-2587	YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK		
NR-141	YLKERITNQV QLLLTHQKSN IEFKLLYKQL NFTENETDNF EVFQKIIDEK		
	748		797