SUPPORTING INFECTIOUS DISEASE RESEARCH

Anthrax Edema Factor (EF), Recombinant from Bacillus anthracis

Catalog No. NR-2585

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

Product Description: Recombinant anthrax edema factor (EF) was produced in a nonsporulating 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.

Lot: 1784C1A

Manufacturing Date: 29SEP2005

TEST	SPECIFICATIONS	RESULTS
Visual Appearance (after reconstitution)	Clear and colorless	Clear and colorless
SDS-PAGE (Coomassie Blue densitometer scan)	 ~ 80–90 kDa band is ≥ 90% of total density 	~ 80–90 kDa band is 99.4% of total density
SDS-PAGE (Silver stain)	Report results	~ 80–90 kDa band is 93.4% of total density
Identification by Electrospray Mass Spectrometry	Report results (expected MW is 88,952 Da)	88,968.8 Da
Functional Activity Adenylate cyclase activity in the presence of 1 μg/mL protective antigen (cAMP accumulation in CHO cells) ^{1,2} Adenylate cyclase activity in cell-free system (cAMP formation from ATP) ^{1,3}	Report results Report results	 4.1 μM cAMP/mg CHO cell protein/mg toxin protein 132 μM cAMP/minute/mg toxin protein
Microbial Content	No detectable colony-forming units in 0.2 mL final product	No detectable colony-forming units in 0.2 mL final product
Endotoxin Content (Limulus Amoebocyte Lysate assay)	< 0.5 EU endotoxin per µg protein	0.024 EU endotoxin per µg protein
Contaminating Protease Detection 6 hour incubation of 1 mg/mL solution at 37°C	Major band is ≥ 85% of initial density	Major band is ≥ 85% of initial density
Absorbance Ratio (OD ₂₈₀ /OD ₂₆₀)	≥ 1.7	1.7
Absorbance Ratio (OD ₂₈₀ /OD ₃₂₀)	≥ 10	21

¹Activity with NR-2585 was comparable to that of BEI Resources NR-141 in side-by-side experiments in October, 2005.

²Kumar, P., et al. "Anthrax Edema Toxin Requires Influx of Calcium for Inducing Cyclic AMP Toxicity in Target Cells." <u>Infect. Immun.</u> 70 (2002): 4997–5007. PubMed: 12183546.

³Hewlett, E. L., et al. "Adenylate Cyclase Toxin from *Bordetella pertussis*. Conformational Change Associated with Toxin Activity." <u>J. Biol. Chem.</u> 266 (1991): 17503–17508. PubMed. 1894634.

Date: 24 MAY 2012

Signature: Dorothy C. young

Title: Technical Manager, BEI Authentication or designee

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