b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-2587

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

Catalog No. NR-2587

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

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

Lot: 1784C1B **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]	Report results	4.1 µM cAMP/mg CHO cell protein/mg toxin protein
Adenylate cyclase activity in cell-free system (cAMP formation from ATP)[1,3]	Report results	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% initial density	Major band is ≥ 85% initial density
Absorbance Ratio (OD_{280}/OD_{260})	≥ 1.7	1.7
Absorbance Ratio (OD_{280}/OD_{320})	≥ 10	29

[1]Activity of NR-2587 was comparable to that of BEI Resources NR-141 in side-by-side experiments in October, 2005.

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

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

Date: 24 MAY 2012 **Signature:** Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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