

Certificate of Analysis for NR-141

Anthrax Edema Factor (EF), Recombinant from Bacillus anthracis

Catalog No. NR-141

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: 1732A1 Manufacturing Date: 06FEB2002

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 95% of total density
Identification by Electrospray Mass Spectrometry	Report results (expected MW is 88,960 Da)	88,988 Da
Functional Activity Adenylate cyclase activity in the presence of 1 μg/mL protective antigen (cAMP accumulation in CHO cells) ^{1,2}	Report results	157 µM cAMP/mg CHO cell protein/mg toxin protein (MAR2003) 10.1 µM cAMP/mg CHO cell protein/mg toxin protein
Adenylate cyclase activity in cell-free system (cAMP formation from ATP) ^{1,3}	Report results	(OCT2005) 2,512 μM cAMP/minute/mg toxin protein (MAR2003) 227 μM cAMP/minute/mg toxin protein (OCT2005)
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) ⁴	< 0.5 EU endotoxin per μg protein	0.15 EU endotoxin per μg protein
Contaminating Protease Detection 3 hour incubation of 1 mg/mL solution at 37°C	Major band is ≥ 85% initial density	Major band is 100% initial density
Absorbance Ratio (OD ₂₈₀ /OD ₂₆₀)	≥ 1.70	1.83
Absorbance Ratio (OD ₂₈₀ /OD ₃₁₀)	≥ 10	14.7

Activity with NR-141 was comparable to that of BEI Resources NR-2585 and NR-2587 in side-by-side experiments in October, 2005.

¹Using BSA as a standard

Date: 24 MAY 2012

Signature:

Title: Technical Manager, BEI Authentication or designee

)crothy C. Young

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²Kumar, P., N. Ahuja, and R. Bhatnagar, R. "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.

⁴Performed by LAL Testing Service, Cambrex BioScience.



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