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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-14869

CFP-10, Recombinant Protein Reference Standard

Catalog No. NR-14869
This reagent is the tangible property of the U.S. Government.

Product Description: NR-14869 is a recombinant form of the culture filtrate antigen, CFP-10, from *Mycobacterium tuberculosis*. The recombinant protein consists of the native protein sequence in addition to a hexa-histidine tag. The recombinant protein was expressed in *Escherichia coli* and purified using standard chromatographic techniques followed by endotoxin removal procedures.

Lot: 62795353 **Manufacturing Date: 19AUG2014**

Production and QC testing were performed by Colorado State University. The Colorado State University documentation for lot 14.rEC.07.21A.HLY.CFP10 is attached.

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.

Support Provided by NIAID

BEI Resources
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Date Production Started: July 21, 2014

Lot Number: 14.rEC.07.21A.HLY.CFP10

Notebook Number and Page Number: HLY ATCC #1 NOTEBOOK, pp. 44-63

Production from Seed Culture/ Clone: no

Production from freshly-transformed Cells: yes

Host Strain used for Gene Expression: *E. coli* BL21 (DE3) pLysS

Recombinant Plasmid possessing the Recombinant Gene: pET-23b

Culture Type? Shake Flask_____ Stationary_____ Fermenter X

Culture Size: 5L

Culture Medium: HyperBroth (Athena Enzyme Systems)

Selection (Antibiotic/ Concentration): $Amp^{100}Cam^{34}$

Time and Temperature of culture prior to Induction: 3:15, 21^0C

Final Concentration of IPTG added for Induction: 0.5 mM

Method for Lysis of Cells: Probe Sonication

Protein Purification Procedures: His-bind Resin Purification

Date Production Finished: August 19, 2014

NOTES ON PURIFICATION:

Cells were sonicated on ice with 60 second bursts followed by 90 second intervals.

His-bind resin purification per Novagen except for additional Endotoxin (ET) removal steps.

ET removal done by washing column with 10 column volumes (CV) of 10 mM Tris-HCl, followed by 10 CV of 0.5% ASB-14. This was again followed by 10 CV of 10 mM Tris-HCl and eluted with 4 CV of 10 mM Tris-HCL+ 1 M Imidazole. All buffers were pH= 8.0. Wash fraction was dialyzed into Bind Buffer and passed over column a second time.

Eluted proteins were exchanged into 10 mM Tris-HCl, pH 8.0 then into 10 mM Ammonium Bicarbonate.

Quality Control

Lot Number: 14.rEC.07.21A.HLY.CFP10

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 0.455 mg/mL

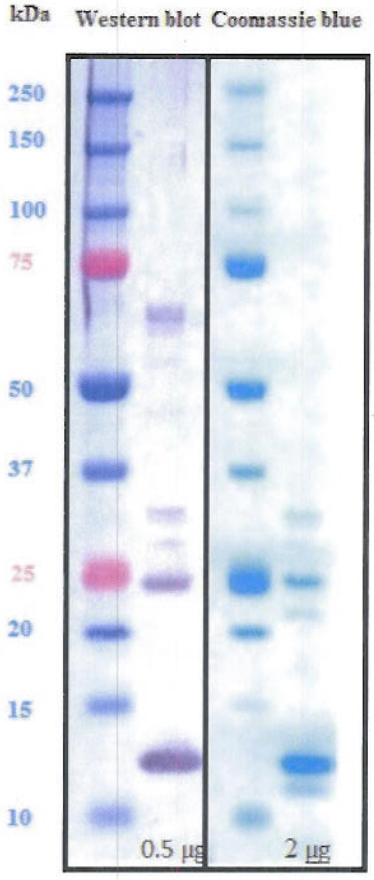
Performed Endotoxin Removal? yes

Endotoxin Contamination: 6.39 ng/mg protein

Purity confirmed by SDS-PAGE and Coomassie blue (see below)

Identity confirmed by Western Blot: X (see below)

Antibody used for Western Blot: α-CFP10 Polyclonal



Aliquot Information: 1 mg × 22 vials, 0.18mg × 1 vial

Producer's Name: _____ Date: 8/22/2014

Supervisor's Name: _____ Date: 8/22/2014