Certificate of Analysis for NR-19335

ML0050/CFP-10 Recombinant Protein from *Mycobacterium leprae*

Catalog No. NR-19335
This reagent is the tangible property of the U.S. Government.

Product Description: NR-19335 is a recombinant form of a culture filtrate protein (ML0050/CFP-10) from *Mycobacterium leprae*. The recombinant His-tagged protein was expressed in *Escherichia coli*, strain BL21(DE3)pLysS and purified using standard chromatographic techniques followed by endotoxin removal procedures. NR-19335 has a molecular weight of approximately 10 kDa.

Lot: 60979815 **Manufacturing Date: 09AUG2013**

Production and QC testing were performed by Colorado State (CSU). The CSU documentation for lot 13.rEC.06.05.coc.MLCFP10 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.

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Recombinant Protein Production and Quality Control Record

Date Production Started: 06/05/2013

Lot Number: 13.rEC.06.05.coc.MLCFP10; BEI lot# 60979815

Notebook Number and Page Number: COC TB #3 NOTEBOOK pp. 47-60; 81-82

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): Kan^{50}

Time and Temperature of culture prior to Induction: 37°C, 3:50

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: 08/09/2013

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 1 CV of 20 mM Tris-HCl + 1 M Imidazole + 6M urea. All buffers were pH= 7.90.

Eluted proteins were exchanged into 10 mM Ammonium Bicarbonate supplemented with 4M urea. A second exchange was done into 10 mM Ammonium Bicarbonate supplemented with 2M urea. Two more exchanges were done into 10mM Ammonium biocarbonate with no urea.

Quality Control

Lot Number: 13.rEC.06.05.coc.MLCFP10; BEI Lot# 60969815 MA 1/14/14

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 10.014 mg/mL

Performed Endotoxin Removal? Yes

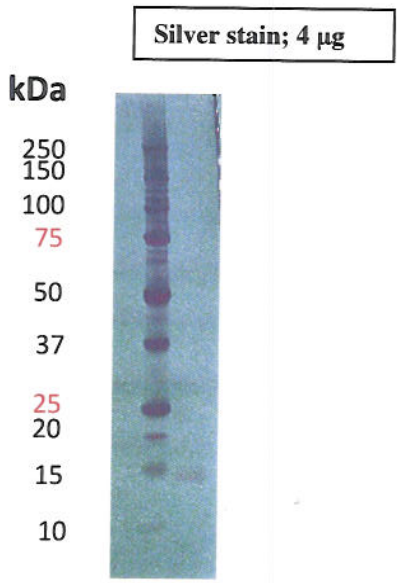
Endotoxin Contamination: 2.320 ng/mg protein

Purity confirmed by SDS-PAGE and Silver Staining (see below)

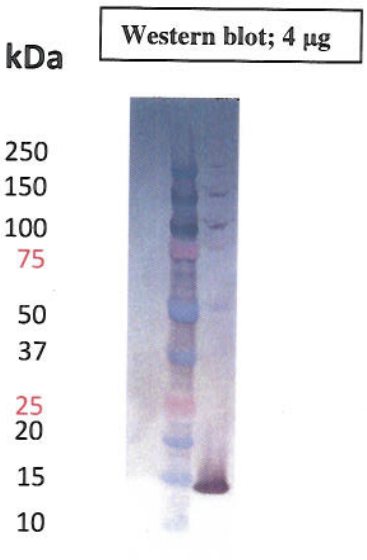
Identity confirmed by Western Blot:__x___ or Mass Spectrometry:_____(see below)

Antibody used for Western Blot: α-poly-Histidine Polyclonal

Silver stain; 4 µg



Western blot; 4 µg



Aliquot Information: 0.5 mg x 30 (to BEI); 0.5 mg x 26 (IHO) and 50.0 mg x 5 (IHO)

Producer's Name: Carma O. Cook Date: 08/21/2013

Supervisor's Name: _____ Date: 8/21/2013