

CFP-10, Recombinant Protein Reference Standard**Catalog No. NR-14869**

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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: 63050383**Manufacturing Date: 26MAY2015**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 15.rEC.03.15.pk.CFP10 is attached.

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Recombinant Protein Production and Quality Control Record

Date Production Started: 05/15/2015

Lot Number: 15.rEC.03.15.pk.CFP10; 63050383

Notebook Number and Page Number: PK bioreactor/fermentor notebook #1 pp.36-55

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): Amp¹⁰⁰Cam³⁴

Time and Temperature of culture prior to Induction: 9:00pm, 37.1C

Final Concentration of IPTG added for Induction: 0.5mM

Method for Lysis of Cells: Probe Sonication

Protein Purification Procedures: His-bind Resin Purification

Date Production Finished: 05/26/2015

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

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

Quality Control

Lot Number: 15.rEC.03.15.pk.CFP10; 63050383

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 0.5mg/ml

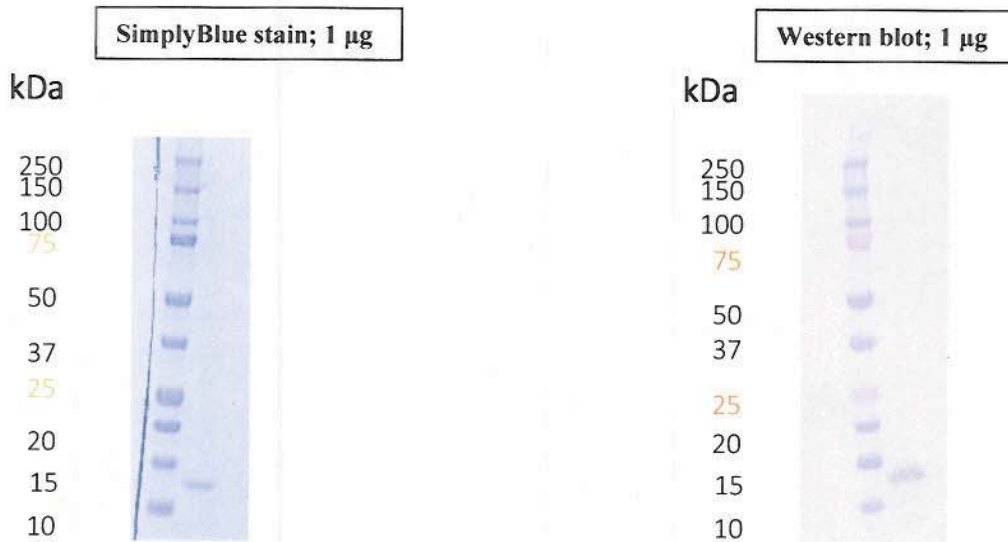
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 0.5ng/mg protein

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

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

Antibody used for Western Blot: α -CFP10 Polyclonal (rabbit)



Aliquot Information: 9 x 1.0 mg

Producer's Name: Philip J. [Signature] Date: 05/26/15
Supervisor's Name: [Signature] Date: 05/27/15