

**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: 63524286****Manufacturing Date: 14MAY2015**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 15.rEC.04.16.pk.CFP10 is attached.

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## Recombinant Protein Production and Quality Control Record

Date Production Started: 04/16/2015

Lot Number: 15.rEC.04.16.pk.CFP10; 63524286

Notebook Number and Page Number: PK bioreactor/fermentor notebook #1 pp.43-55

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): Amp<sup>100</sup>Cam<sup>34</sup>

Time and Temperature of culture prior to Induction: 345pm, 37C

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: 05/14/2015

### 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.04.16.pk.CFP10; 63524286

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 0.8mg/ml

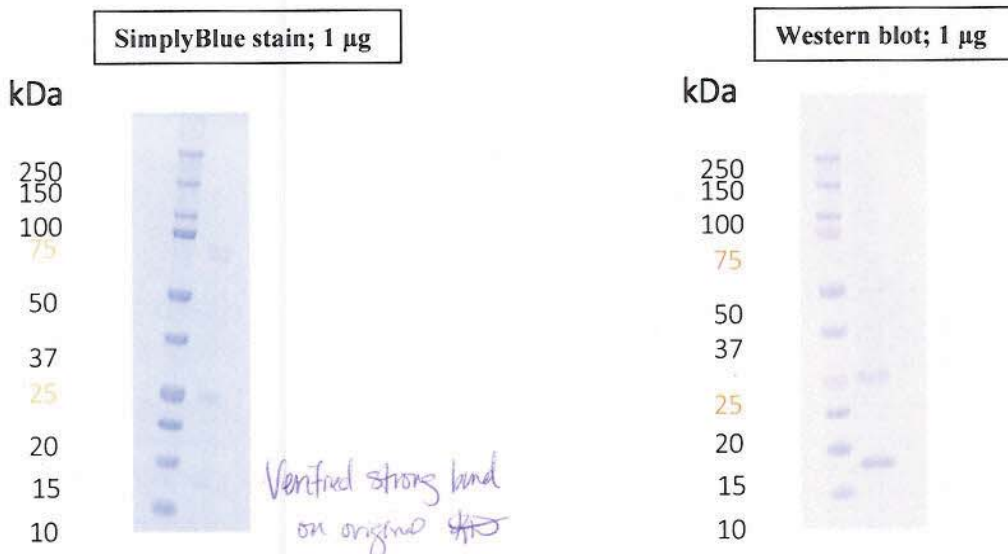
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 7.9ng/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:  $\alpha$ -CFP10 Polyclonal (rabbit)



Aliquot Information: 10 x 1.0 mg

Producer's Name: *Philippe K...* Date: 05/26/15  
Supervisor's Name: *Spencer D...* Date: 5/27/15