

Mpt64, Recombinant Protein Reference Standard**Catalog No. NR-44102**

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Product Description: NR-44102 is a recombinant form of the secreted immunogenic protein Mpt64 (also referred to as mpb64) from *Mycobacterium tuberculosis*. The recombinant protein was expressed in *Escherichia coli* and purified using standard chromatographic techniques followed by endotoxin removal procedures.

Lot: 61983710**Manufacturing Date: 26APR2013**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 13.rEC.04.17.coc.MtbMpt64 is attached.

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Recombinant Protein Production and Quality Control Record

Date Production Started: 04/17/2013

Lot Number: 13.rEC.04.17.coc.MtbMpt64

Notebook Number and Page Number: COC TB #2 NOTEBOOK pp. 109-116

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

Culture Size: 5L

Culture Medium: HyperBroth (Athena Enzyme Systems)

Selection (Antibiotic/ Concentration): Amp¹⁰⁰Cam³⁴

Time and Temperature of culture prior to Induction: 3:00, 37.1°C

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: 04/26/2013

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 4 CV of 10 mM Tris-HCl + 1 M Imidazole. All buffers were pH= 8.0

Eluted proteins were exchanged into 10 mM Ammonium Bicarbonate.

Quality Control

Lot Number: 13.rEC.04.17.coc.MtbMpt64

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 12.409 mg/mL

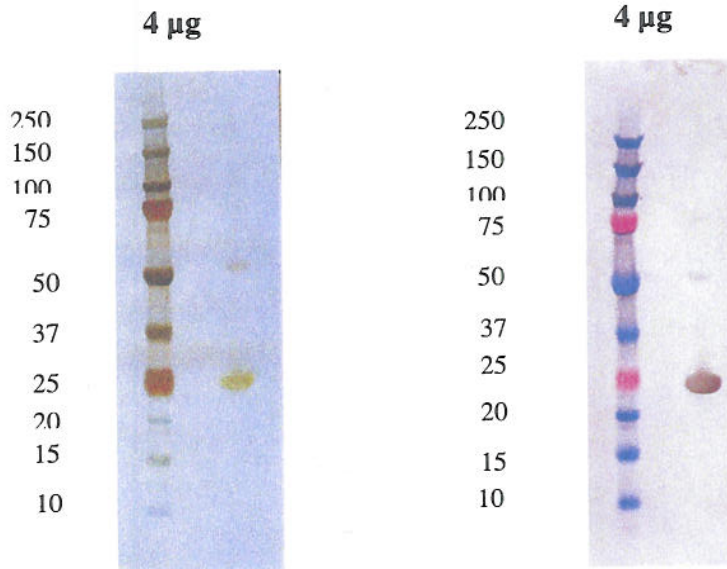
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 6.38 ng endotoxin/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: anti-poly-His mouse monoclonal



Aliquot Information: 50 x 1 mg; 6 x 5 mg; 5 x 50 mg (330 mg total)

Producer's Name: Carma D. Cook Date: 05/02/2013

Supervisor's Name: [Signature] Date: 05/2/2013