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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-31384

HspX, Recombinant Protein Reference Standard

Catalog No. NR-31384
This reagent is the tangible property of the U.S. Government.

Product Description: NR-31384 is a recombinant form of the heat shock protein, HspX from *Mycobacterium tuberculosis*. The recombinant protein was expressed in *Escherichia coli* and purified using standard chromatographic techniques followed by endotoxin removal procedures.

Lot: 61056065 **Manufacturing Date: 29JUN2011**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for lot 11.rEC.5.31.hly.HspX (pre-vial) 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

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HHSN272201000027C

Recombinant Protein Production and Quality Control Record

Date Production Started: May 31, 2011

Lot Number: 11.rEC.5.31.hly.HspX

Notebook Number and Page Number: HLYang Contract #1 Notebook, pp. 59-76

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

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

Time and Temperature of culture prior to Induction: 2:41 / 35.9°C

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: June 29, 2011

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 for 2 times.

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Quality Control

Lot Number: 11.rEC.5.31.hly.HspX

Method for Determining Protein Concentration: BCA Assay (Pierce)

Final Protein Concentration: 3.53 mg/mL

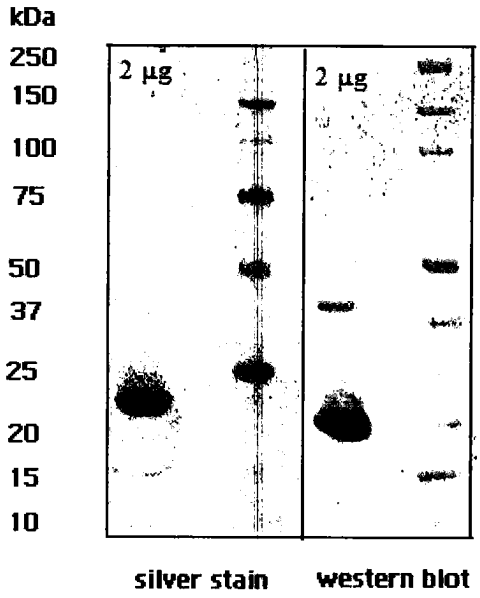
Performed Endotoxin Removal? yes

Endotoxin Contamination: 0.003 ng/mg

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: CS-49



Aliquot Information: 8 x 50mg*

*Aliquot information reflects aliquots made at time of QC. 1mg aliquots were subsequently made for distribution.

Producer's Name: _____ Date: 5/3/2012

Supervisor's Name: _____ Date: 5/4/2012