Certificate of Analysis for NR-14868

ESAT-6 Recombinant Protein Reference Standard

Catalog No. NR-14868
This reagent is the tangible property of the U.S. Government.

Product Description: NR-14868 is a recombinant form of the early secretory antigenic target protein, ESAT-6. The protein sequence consists of amino acid residues 1 to 103 including a hexa-histidine tag at the C-terminus. The recombinant protein was expressed in *Escherichia coli* and purified using standard chromatographic techniques followed by endotoxin removal procedures. NR-14868 has a theoretical molecular weight of approximately 11 kDa.

Lot: 61202620 **Manufacturing Date: 03MAR2008**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for bulk lot 10.rEC.1.19.ks.b.ESAT6 is attached. This lot was aliquoted at ATCC®.

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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Recombinant Protein Production and Quality Control Record for TBRMVT Contract HHSN266200400091C

Date Production Started: 1 February 2008

Lot Number: 10.rEC.1.19.ks.b.ESAT6

Notebook Number and Page Number: KES TB #5 Notebook, pp. 81-85

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 ES)

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

Time and Temperature of culture prior to Induction: 270 min, 22.7^0C

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: 3 March 2008

NOTES ON PURIFICATION:

Cells were sonicated on ice with 60 second bursts followed by 90 second intervals. Some of Lysate was frozen, and 80ml was used for this purification.

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.

Amino Acid Sequence

mteqqwnfagieaaasaiqgnvtsihslldegkqsltklaaawggsgseayqgvqqkwdatatelnnalqnlartiseagqa
mastegnvtgmfalehhhhhh

Quality Control

Lot Number: 10.rEC.1.19.ks.b.ESAT6

Method for Determining Protein Concentration: BCA assay (Pierce)

Final Protein Concentration: 5.5 mg/ml Total Protein: 504 mg

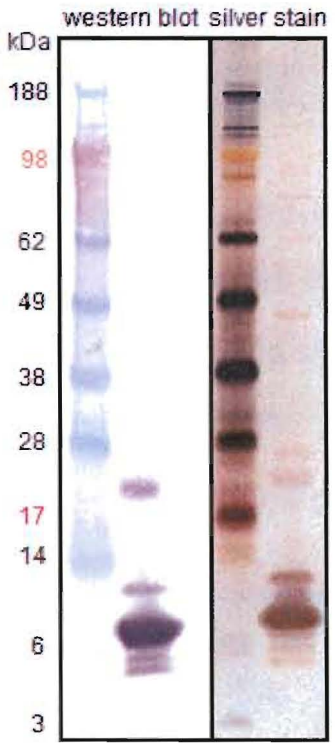
Performed Endotoxin Removal? yes

Endotoxin Contamination: 0.12 ng/mg protein, verified by LAL assay

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-Esat6 rabbit polyclonal



Aliquot Information:
20 x 20 mg ; 15 x 5 mg ; 29 x 1 mg

Producer's Name: Katie Straus Date: 1/29/2010

Supervisor's Name: [signature] Date: 2/1/2010