

ESAT-6 Recombinant Protein Reference Standard**Catalog No. NR-14868**

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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 hexahistidine 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: 61602193**Manufacturing Date: 15JUN2013**

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 13.rEC.05.21.coc.MtbEsat6 is attached.

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Recombinant Protein Production and Quality Control Record

Date Production Started: 05/21/2013

Lot Number: 13.rEC.05.21.coc.MtbEsat6; ATCC# 61602193

Notebook Number and Page Number: COC TB #3 NOTEBOOK pp. 35-46

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: 5:00, 37.0°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: 06/15/2013

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= 7.90 (with the exception of the elution buffer at pH 8.0)

Eluted proteins were exchanged into 10 mM Ammonium Bicarbonate.

Quality Control

Lot Number: 13.rEC.05.21.coc.MtbEsatt6; ATCC# 61602193

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 3.662 mg/mL

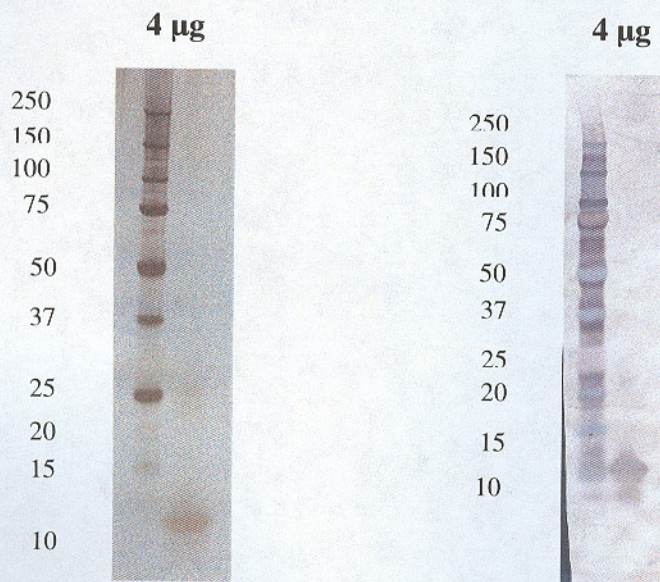
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 8.361 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: 28 x 1 mg

Producer's Name: Carma O. Cook Date: 06/15/2013

Supervisor's Name: [Signature] Date: 6/17/2013