
Ag85B Recombinant Protein Reference Standard

Catalog No. NR-14870

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Product Description: NR-14870 is a recombinant form of the antigen 85 complex B (Ag85B) protein. 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: 61792968

Manufacturing Date: 27JAN2014

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 13.rEC.12.19.coc.MtbAg85b is attached.

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Recombinant Protein Production and Quality Control Record

Date Production Started: 12/19/2013

Lot Number: 13.rEC.12.19.coc.MtbAg85b; 61792968

Notebook Number and Page Number: COC TB #4 NOTEBOOK pp. 4 -27

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: 01/27/2014

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 5 CV of 10 mM Tris-HCl + 1 M Imidazole + 6M urea. All buffers were pH= 8.0

Eluted proteins were exchanged into 10 mM Ammonium Bicarbonate + 4M urea; then into 10 mM Ammonium Bicarbonate + 2M urea; and finally into 10 mM Ammonium Bicarbonate .

Quality Control

Lot Number: 13.rEC.12.19.coc.MtbAg85b; 61792968

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 2.644 mg/mL

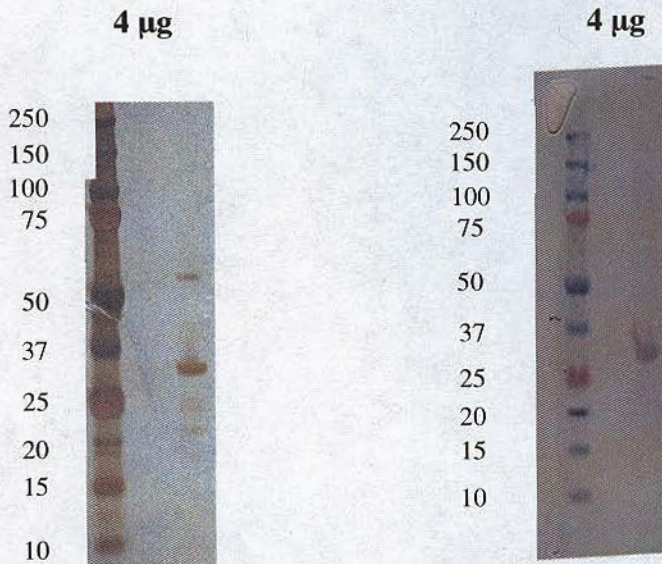
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 10.75 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: IT-44 mouse monoclonal



Aliquot Information: 23 x 1 mg; 1 x 0.8 mg

Producer's Name: Carmal O. Croft Date: 01/28/2014

Supervisor's Name: [Signature] Date: 1/28/2014