

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: 62024486**Manufacturing Date: 25SEP2014**

Production and QC testing were performed by Colorado State University (CSU). The CSU documentation for lot 14.rEC.09.02.HLY.Ag85B is attached.

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Date Production Started: September 2, 2014

Lot Number: 14.rEC.09.02.HLY.Ag85B

Notebook Number and Page Number: HLY ATCC #1 NOTEBOOK, pp. 65-75

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: 4L

Culture Medium: HyperBroth (Athena Enzyme Systems)

Selection (Antibiotic/ Concentration): Amp¹⁰⁰ Cam³⁴

Time and Temperature of culture prior to Induction: 3:05, 22 °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: September 25, 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 4 CV of 10 mM Tris-HCL+ 1 M Imidazole. All buffers were pH= 8.0. Wash fraction was dialyzed into Bind Buffer and passed over column a second time.

Eluted proteins were exchanged into 10 mM Tris-HCl, pH 8.0 then into 10 mM Ammonium Bicarbonate.

Quality Control

QC: 14.rEC.09.02.HLY.Ag85B

Lot Number: 14.rEC.09.02.HLY.Ag85B

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 1.33 mg/mL

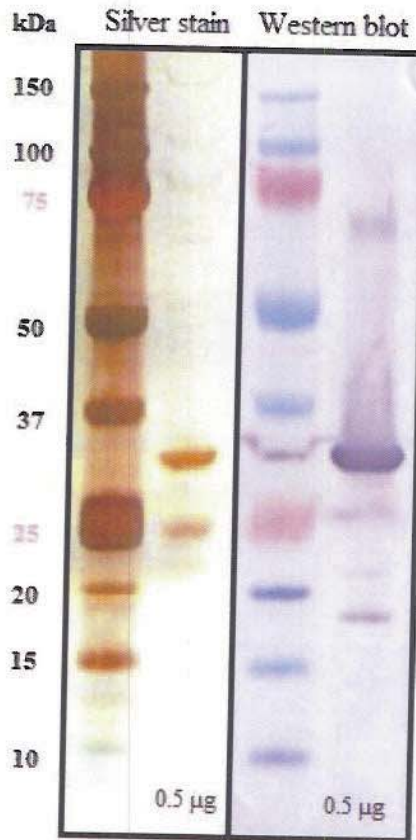
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 0.73ng/mg protein

Purity confirmed by SDS-PAGE and Silver Staining (see below)

Identity confirmed by Western Blot: X and Silver stain: X (see below)

Antibody used for Western Blot: Ag85 Polyclonal



Aliquot Information: 1 mg × 103 vials, 0.5 mg × 1 vial, 0.4 mg × 1 vial

Producer's Name: _____

Irony Liangyan

Date: _____

9/26/2014

Supervisor's Name: _____

[Signature]

Date: _____

9/29/2014