

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: 62187650**Manufacturing Date: 23MAY2011**

Production and QC testing were performed by Colorado State University. The Colorado State University documentation for bulk lot 11.rEC.4.4.hly.Ag85B is attached. The bulk lot was received as a lyophilized powder from CSU and was rehydrated in 10 mM ammonium bicarbonate at ATCC. Approximately 1 mL of a 1 mg/mL solution was dispensed per vial, yielding a final amount of 1 mg per vial.

Note: Lot 62187650 is provided as a frozen liquid and not as a lyophilized solid as were previous lots of NR-14870.

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HHSN272201000027C

Recombinant Protein Production and Quality Control Record

General Information

BEI Catalog number: NR-14870

ATCC Lot number: 60209908

CSU Lot Number: 11.rEC.4.4.hly.Ag85B

Production Information

Date Production Started: April 4, 2011

Notebook Number and Page Number: Business HLY #1, p. 21-55

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 _____ Fermentor X

Culture Size: 5L

Culture Medium: HyperBroth (Athena Enzyme Systems)

Selection (Antibiotic/ Concentration): Amp¹⁰⁰Cam³⁴

Time and Temperature of culture prior to Induction: 3:38, 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: May 23, 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 5 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 Ammonium Bicarbonate.

Quality Control

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 0.387 mg/mL

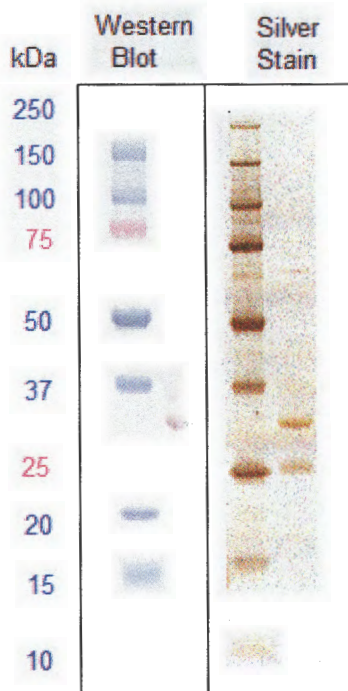
Performed Endotoxin Removal? yes

Endotoxin Contamination: 7.14 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: IT-44 α -Ag85 Monoclonal



Aliquot Information: 1 × 50.3 mg

Producer's Name: *Hongliangyang* Date: 01/12/2012

Supervisor's Name: *[Signature]* Date: 01/13/2012