

**Ag85A Recombinant Protein Reference Standard****Catalog No. NR-14871**

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**Product Description:** NR-14871 is a recombinant form of the antigen 85 complex A (Ag85A) protein. The recombinant protein consists of the native protein sequence in addition to a hexahistidine tag. The recombinant protein was expressed in *Escherichia coli* and purified using standard chromatographic techniques followed by endotoxin removal procedures.

**Lot: 61056243****Manufacturing Date: 14MAY2010**

QC testing was performed by Colorado State University under the TB Vaccine Testing and Research Materials Contract (NIH). The Colorado State University documentation for the pre-vial bulk lot 10.rEC.05.03.ks.Ag85A is attached.

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**Recombinant Protein Production and Quality Control Record  
for TBRMVT Contract HHSN266200400091C**

Date Production Started: May 3, 2009

Lot Number: 10.rEC.05.03.ks.Ag85A

Notebook Number and Page Number: KEE TB #6 Notebook, pp. 1-7

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 15b

Culture Type? Shake Flask        Stationary        Fermenter   X  

Culture Size: 5 L

Culture Medium: HyperBroth (Athena Enzyme Systems)

Selection (Antibiotic/ Concentration): Amp<sup>100</sup>Cam<sup>34</sup>

Time and Temperature of culture prior to Induction: 221 min, 21<sup>0</sup>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 14, 2010

**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

Eluted proteins were exchanged into 10 mM Ammonium Bicarbonate.

## Quality Control

Lot Number: 10.rEC.05.03.ks.Ag85A

Method for Determining Protein Concentration: BCA (Pierce)

Final Protein Concentration: 2.86 mg/mL

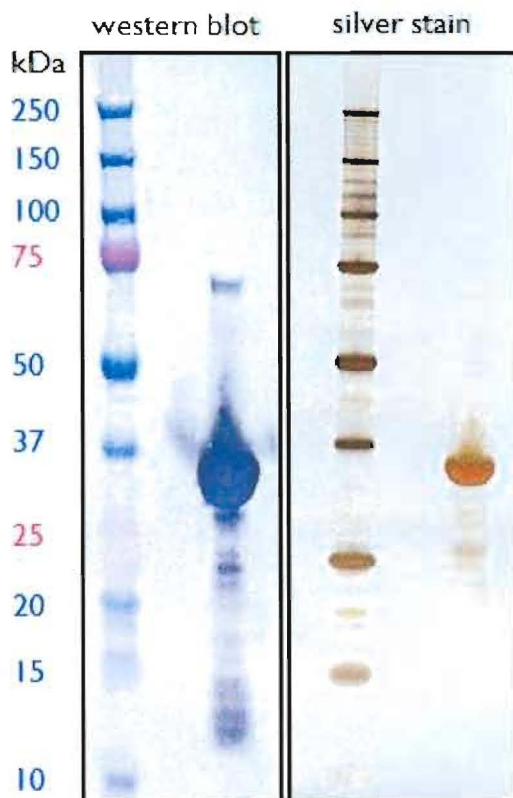
Performed Endotoxin Removal? Yes

Endotoxin Contamination: 0.14 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:  $\alpha$ -Ag85 rabbit polyclonal



Aliquot Information:

1 x 100 mg ; 10 x 10 mg ; 14 x 1 mg    Total = 214 mg in inventory

Producer's Name: Kate Armin      Date: 5/14/10

Supervisor's Name: [Signature]      Date: 5/21/2010