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 hexa-histidine 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$^{106}$Cam$^{34}$
Time and Temperature of culture prior to Induction: 221 min, 21$^\circ$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: α-Ag85 rabbit polyclonal

![](western blot and silver stain image)

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

Producer’s Name: ___________________________ Date: 5/14/10

Supervisor’s Name: __________________________ Date: 5/21/2010