

## Certificate of Analysis for NR-49427

## Ag85A, Recombinant Protein Reference Standard

#### Catalog No. NR-49427

This reagent is the tangible property of the U.S. Government.

### **Product Description:**

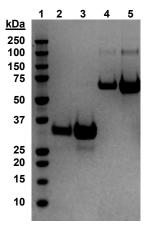
NR-49427 is a recombinant form of the antigen 85 complex A (Ag85A) protein. The recombinant protein consists of the native protein sequence in addition to a hexa-histidine tag. The recombinant protein was expressed in *Escherichia coli* (*E. coli*) BL21 (DE3) pLysS cells and purified by immobilized-metal affinity chromatography. Ag85A was harvested and purified from *E. coli* BL21 (DE3) pLysS cells transformed with BEI Resources NR-13292 lot 04.EC.09.03.

Lot: 70032803 Manufacturing Date: 10AUG2020

TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	White powder
SDS-PAGE (Coomassie Blue Stain)	Dominant band ~ 35 kDa > 90% pure	Dominant band of ~ 35 kDa accounts for > 90% of total staining intensity (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay <sup>1</sup>	Report results	0.8 mg per mL
Final Product		
Quantity per vial	Report results	0.4 mg
Volume per vial	Report results	500 μL
Western Blot Analysis		
Polyclonal anti-Ag85A <sup>2</sup>	Reactive	Reactive (Figure 2)
Monoclonal anti-histidine tag <sup>3</sup>	Reactive	Reactive (Figure 3)
Endotoxin Content		
(Limulus Amoebocyte Lysate Assay)	1 to 10000 EU per mg protein	57.4 EU per mg protein
Sterility	0.2 µm sterile-filtered	0.2 µm sterile-filtered

<sup>&</sup>lt;sup>1</sup>Using bovine serum albumin (BSA) as a standard

Figure 1: SDS-PAGE of Ag85A



Lane 1: Precision Plus Protein™ All Blue Protein Standard

Lane 2: NR-49427, 2.5 µg Lane 3: NR-49427, 8 µg Lane 4: BSA, 2.5 µg Lane 5: BSA, 8 µg

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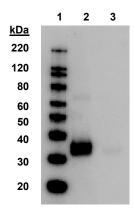
<sup>&</sup>lt;sup>2</sup>Rabbit anti-Ag85A polyclonal antibody (Abcam, ab193499) (1:1000 dilution)

<sup>&</sup>lt;sup>3</sup>Anti-histidine monoclonal antibody (R&D Systems, MAB050) (1:1000 dilution)



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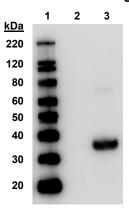
Figure 2: Western Blot with Polyclonal Anti-Ag85A



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-49427, 1 μg Lane 3: BSA, 1 μg

Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard

Lane 2: BSA, 1 μg Lane 3: NR-49427, 1 μg

/Heather Couch/

BEI Resources www.beiresources.org

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Program Manager or designee, ATCC Federal Solutions

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