

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* BL21 (DE3) pLysS cells and purified by immobilized-metal affinity chromatography.

Lot¹: 70021268

Manufacturing Date: 21FEB2019

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 Assay²	Report results	1.06 mg per mL
Final Product Quantity per vial Volume per vial	Report results Report results	1.06 mg 1 mL
Western Blot Analysis Polyclonal anti-Ag85A ³ Monoclonal anti-histidine tag ⁴	Reactive Reactive	Reactive (Figure 2) Reactive (Figure 3)
Endotoxin Content (Limulus Amoebocyte Lysate Assay)	1 to 10000 EU per mg protein	< 4.725 EU per mg protein
Sterility	0.2 µm sterile-filtered	0.2 µm sterile-filtered

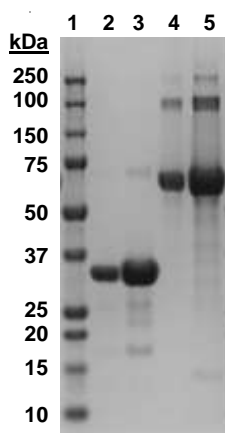
¹Ag85A was harvested and purified from *E. coli* BL21 (DE3) pLysS cells transformed with BEI Resources NR-13292 lot 04.EC.09.03.

²Using bovine serum albumin (BSA) as a standard

³Rabbit anti-Ag85A polyclonal antibody (Abcam, ab193499) (1:1000 dilution)

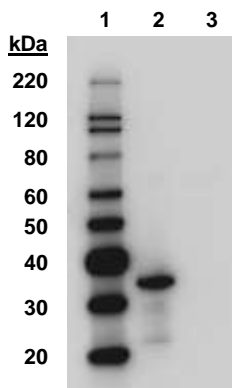
⁴Anti-histidine monoclonal antibody (R&D Systems, MAB050) (1:1000 dilution)

Figure 1: SDS-PAGE of Ag85A



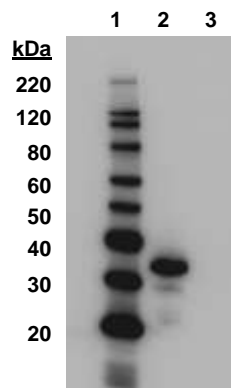
Lane 1: Precision Plus Protein™ All Blue Protein Standard
Lane 2: NR-49427, 2.5 µg
Lane 3: NR-49427, 10 µg
Lane 4: BSA, 2.5 µg
Lane 5: BSA, 10 µg

Figure 2: Western Blot with Polyclonal Anti-Ag85A



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-49427, 2 µg
Lane 3: BSA, 1 µg

Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-49427, 2 µg
Lane 3: BSA, 1 µg

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

17 MAY 2019

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