

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

Lot¹: 64001335 Manufacturing Date: 18MAR2016

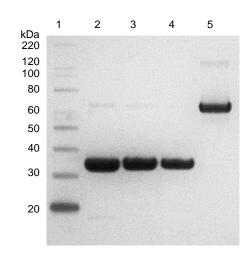
TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	White powder
SDS-PAGE (Coomassie Blue Stain)	Dominant band ~ 32 kDa > 90% pure	Dominant band of ~ 32 kDa accounts for > 90% of total staining intensity (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay ²	Report results	1.16 mg per mL
Final Product Quantity per vial Volume per vial	Report results Report results	1.16 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/mg	< 0.755 EU/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.

Figure 1: SDS-PAGE of Ag85A

Lane 1: MagicMark™ XP Protein Standard Lane 2: NR-49427, 10 µg

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



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²Using BSA as a standard

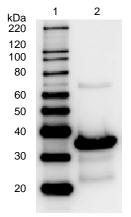
³Rabbit anti-Ag85A polyclonal antibody (Abcam, ab193499) (1:1000 dilution)

⁴6xHis tag monoclonal antibody (R&D Systems, MAB050) (1:1000 dilution)



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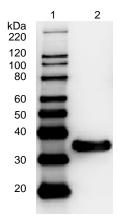
Figure 2: Western Blot with Polyclonal Anti- Ag85A



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-49427, 2.0 µg

Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-49427, 2.0 µg

Date: 24 JUN 2016 Signature:

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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