

Ag85B, Recombinant Protein Reference Standard

Catalog No. NR-49426

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

Product Description:

NR-49426 is a recombinant form of the antigen 85 complex B (Ag85B) 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 transformed with BEI Resources NR-13298 lot 70063217 and purified by immobilized-metal affinity chromatography.

Lot: 70066556

Manufacturing Date: 28JUN2024

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TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	Clear and colorless
SDS-PAGE Analysis	Protein band of interest represents > 90% of total staining intensity	Dominant band of ~ 35 kDa accounts for > 92% of total staining intensity (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay¹	Report results	400 µg/µL
Final Product Quantity per vial Volume per vial	Report results Report results	100 µg 250 µL
Identification by Western Blot Analysis Monoclonal anti-Ag85A tag ^{2,3} Monoclonal anti-histidine tag ⁴	Reactive Reactive	Reactive (Figure 2) Reactive (Figure 3)
Endotoxin Content Limulus Amoebocyte Lysate Assay ⁵	Report results	28.5 EU/mg
Sterility	0.2 µm filter-sterilized	0.2 µm filter-sterilized

¹Using bovine serum albumin (BSA) as a standard

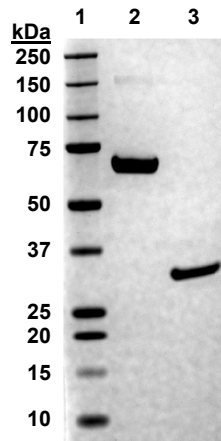
²Using a 1:3000 dilution of rabbit polyclonal anti-*Mycobacterium tuberculosis* Ag85A (Abcam ab193499) as primary antibody and a 1:3000 dilution of HRP-conjugated anti-rabbit IgG (R&D Systems, HAF008) as secondary antibody.

³Members of the Ag85 Complex exhibit extensive cross-reactivity (Wiker, H. G. and M. Harboe. "The Antigen 85 Complex: A Major Secretion Product of *Mycobacterium tuberculosis*." Microbiol. Rev. 56 (1992): 648-661. PubMed: 1480113.).

⁴Using a 1:3000 dilution of mouse monoclonal anti-His (R&D Systems MAB050) as primary antibody and a 1:3000 dilution of HRP-conjugated anti-mouse IgG (R&D Systems HAF007) as secondary antibody

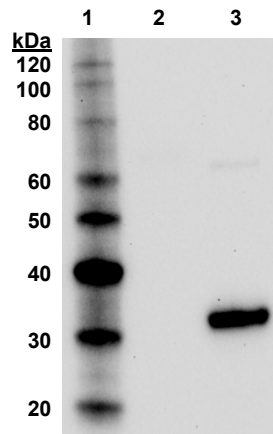
⁵Endosafe®-PTS Cartridge, Charles River Laboratories, Inc., PTS2005F

Figure 1: SDS-PAGE Analysis



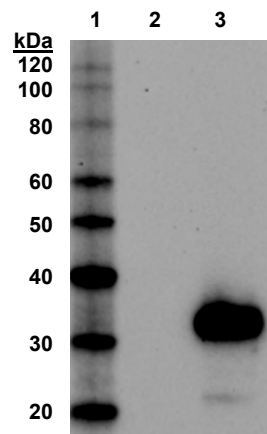
Lane 1: Precision Plus Protein™ All Blue Protein Standard
Lane 2: BSA, 2 µg
Lane 3: NR-49426, 2 µg

Figure 2: Western Blot with Monoclonal Anti-Ag85B



Lane 1: MagicMark™ XP Protein Standard
Lane 2: BSA, 1 µg
Lane 3: NR-49426, 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-49426, 1 µg

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Technical Manager or designee, ATCC Federal Solutions

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