

Certificate of Analysis for NR-49431

ML2028/Ag85B Recombinant Protein from Mycobacterium leprae

Catalog No. NR-49431

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

Product Description:

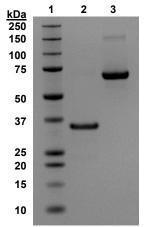
NR-49431 is a recombinant form of the antigen 85B protein (ML2028/Ag85B) [also known as fibronectin-binding protein B (FbpB)] from *Mycobacterium leprae*. The recombinant His-tagged protein was expressed in *Escherichia coli*, strain BL21(DE3)pLysS transformed with BEI Resources NR-19392 lot 61002001 and purified by immobilized-metal affinity chromatography.

Lot: 70055160 Manufacturing Date: 10NOV2022

TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	Colorless and clear
SDS-PAGE Analysis	Protein band of interest represents > 90% of total staining intensity	Dominant band of ~ 35 kDa accounts for 91.6% of total staining intensity (Figure 1)
Concentration by Bicinchoninic Acid Assay Bovine Serum Albumin (BSA; standard)	Report results	1.12 mg/mL
Final Product		
Quantity per vial	Report results	0.223 mg
Volume per vial	Report results	200 μL
Western Blot Analysis		
Polyclonal Anti-Ag85 ¹	Reactive	Reactive (Figure 2)
Monoclonal anti-histidine tag ²	Reactive	Reactive (Figure 3)
Endotoxin Content		
Limulus Amoebocyte Lysate Assay³	Report results	16.56 EU/mg
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

¹Using a 1:4000 dilution of rabbit polyclonal anti-*Mycobacterium tuberculosis* Antigen 85 Complex (Ag85; FbpA/FbpB/FbpC; Genes Rv3804c, Rv1886c, Rv0129c) (BEI Resources NR-13800) as primary antibody and a 1:2000 dilution of HRP-conjugated anti-rabbit IgG (R&D Systems, HAF008) as secondary antibody.

Figure 1: SDS-PAGE Analysis



Lane 1: Precision Plus Protein™ All Blue Protein Standard

Lane 2: NR-49431, 2 μg

Lane 3: BSA, 2 µg

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²Using a 1:4000 dilution of mouse monoclonal anti-6×His (Takara Bio, Inc., 631212) as primary antibody and a 1:2000 dilution of HRP-conjugated anti-mouse IgG (R&D Systems, HAF007) as secondary antibody.



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Figure 2: Western Blot with Polyclonal Anti-Ag85

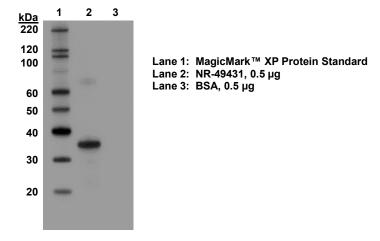
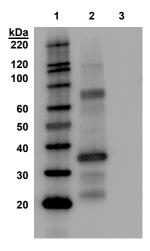


Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-49431, 0.5 μg Lane 3: BSA, 0.5 μg

/Sonia Bjorum Brower/ Sonia Bjorum Brower

21 FEB 2024

Technical Manager or designee, ATCC Federal Solutions

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