

Certificate of Analysis for NR-49424

ESAT-6, Recombinant Protein Reference Standard

Catalog No. NR-49424

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

Product Description:

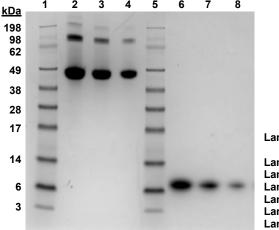
NR-49424 is a recombinant form of the early secretory antigenic target protein, ESAT-6. The protein sequence consists of amino acid residues 1 to 103 including a hexa-histidine tag at the C-terminus. The recombinant ESAT-6 protein was expressed in Escherichia coli BL21 (DE3) pLysS cells transformed with BEI Resources NR-50170 lot 64034836 and purified by immobilized-metal affinity chromatography and lyophilized. ESAT-6 has a theoretical molecular weight of approximately 11 kDa.

Lot: 70016782 Manufacturing Date: 10AUG2018

TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	White, fluffy powder
SDS-PAGE	Protein band of interest represents > 90% of total staining intensity	Dominant band of ~ 10 kDa accounts for > 90% of total staining intensity (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay ¹	Report results	1.2 mg per mL
Final Product Quantity per vial	Report results	1.2 mg
Volume per vial	Report results	1 mL
Western Blot Analysis Polyclonal anti-ESAT-6 ² Monoclonal anti-histidine tag ³	Reactive Reactive	Reactive (Figure 2) Reactive (Figure 3)
Endotoxin Content Limulus Amoebocyte Lysate Assay ⁴	Report results	12.7 EU/mg protein
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

¹Using bovine serum albumin (BSA) as a standard

Figure 1: SDS-PAGE and Silver Stain



Lane 1: SeeBlue® Plus2 Protein Standard

(Invitrogen™, LC5925)

Lane 2: BSA, 4 µg Lane 3: BSA, 2 µg Lane 4: BSA, 1 µg Lane 5: NR-49424, 4 µg Lane 6: NR-49424, 2 µg

Lane 7: NR-49424, 1 µg

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BEI Resources

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²Polycional Anti-Mycobacterium tuberculosis ESAT-6 (antiserum, Rabbit) (BEI Resources NR-13803) (1:1000 dilution)

³His Tag Antibody (R&D Systems, MAB050) (1:1,000 dilution)

⁴Endosafe[®] nexgen-PTS™ (Charles River, PTS20F)



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Figure 2: Western Blot with Polyclonal Anti-ESAT-6

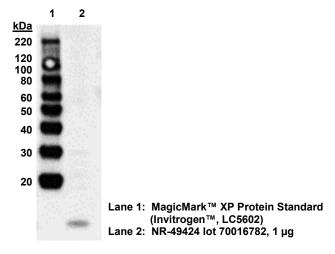
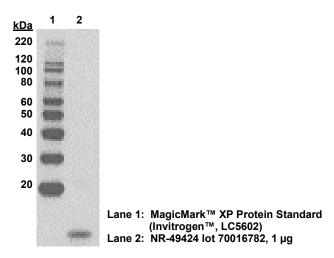


Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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