SUPPORTING INFECTIOUS DISEASE RESEARCH

### 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. The bulk protein was stored at -20°C as NR-49424 lot 70016782. NR-49424 bulk lot 70016782 was thawed and aliquoted on 15NOV2019 to produce this lot.

#### Lot: 70030396

#### Manufacturing Date: 15NOV2019

TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	Lyophilized
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 <sup>1</sup>	Report results	1.06 mg per mL
Final Product		
Quantity per vial	Report results	1.06 mg
Volume per vial	Report results	1 mL
Western Blot Analysis		
Polyclonal anti-ESAT-6 <sup>2</sup>	Reactive	Reactive (Figure 2)
Monoclonal anti-histidine tag <sup>3</sup>	Reactive	Reactive (Figure 3)
Endotoxin Content		
Limulus Amoebocyte Lysate Assay <sup>4</sup>	Report results	17.3 EU/mg protein
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

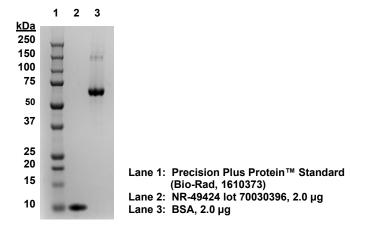
<sup>1</sup>Using bovine serum albumin (BSA) as a standard

<sup>2</sup>Polyclonal Anti-*Mycobacterium tuberculosis* ESAT-6 (antiserum, Rabbit) (BEI Resources NR-13803) (1:1000 dilution)

<sup>3</sup>His Tag Antibody (R&D Systems, MAB050) (1:1000 dilution)

<sup>4</sup>Charles River Endosafe<sup>®</sup>-PTS Endotoxin Testing System (Charles River, PTS200SF)

#### Figure 1: SDS-PAGE and Silver Stain

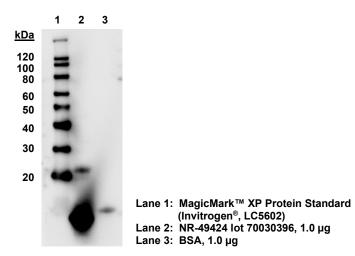


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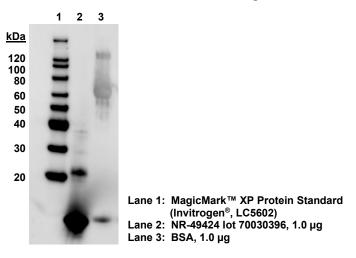
# **Certificate of Analysis for NR-49424**

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







## /Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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