

**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 protein was expressed in *Escherichia coli* (*E. coli*) BL21 (DE3) pLysS cells and purified by immobilized-metal affinity chromatography. ESAT-6 has a theoretical molecular weight of approximately 11 kDa.

**Lot<sup>1</sup>: 70009452**

**Manufacturing Date: 06OCT2017**

TEST	SPECIFICATIONS	RESULTS
<b>Appearance</b>	Report results	White, fluffy powder
<b>SDS-PAGE (Coomassie Blue Stain)</b>	Dominant band ~ 10 kDa > 90% pure	Dominant band of ~ 10 kDa accounts for > 90% of total staining intensity (Figure 1)
<b>Concentration by Bicinchoninic Acid Protein Assay<sup>2</sup></b>	Report results	1.26 mg per mL
<b>Final Product</b> Quantity per vial Volume per vial	Report results Report results	1.26 mg 1 mL
<b>Western Blot Analysis</b> Polyclonal anti-ESAT-6 <sup>3</sup> Monoclonal anti-histidine tag <sup>4</sup>	Reactive Reactive	Reactive (Figure 2) Reactive (Figure 3)
<b>Endotoxin Content</b> Limulus Amoebocyte Lysate Assay <sup>5</sup>	1 to 10000 EU/mg	< 4.00 EU/mg protein
<b>Sterility</b>	0.2 µm sterile-filtered	0.2 µm sterile-filtered

<sup>1</sup>ESAT-6 was harvested and purified from *E. coli* BL21 (DE3) pLysS cells transformed with BEI Resources NR-50170 lot 64034836.

<sup>2</sup>Using BSA as a standard

<sup>3</sup>Rabbit anti-ESAT-6 polyclonal antibody (BEI Resources, NR-13803) (1:1000 dilution)

<sup>4</sup>6xHis tag monoclonal antibody (R&D Systems, MAB050) (1:1000 dilution)

<sup>5</sup>Endosafe<sup>®</sup> nexgen-PTS<sup>™</sup> (Charles River, PTS20F)

**Figure 1: SDS-PAGE of ESAT-6**

Lane 1: Precision Plus Protein<sup>™</sup> All Blue Standard  
 Lane 2: BSA, 1 µg  
 Lane 3: BSA, 2 µg  
 Lane 4: BSA, 4 µg  
 Lane 5: NR-49424, 1 µg  
 Lane 6: NR-49424, 2 µg  
 Lane 7: NR-49424, 4 µg

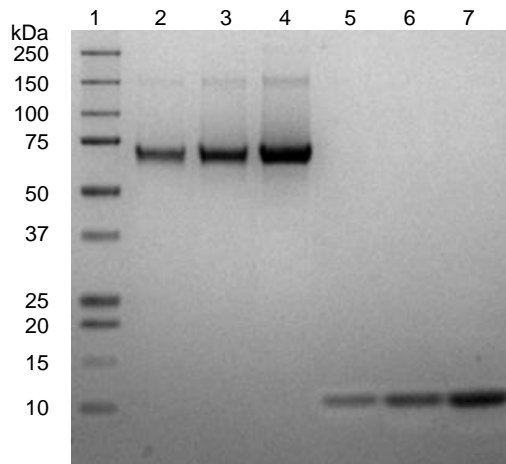
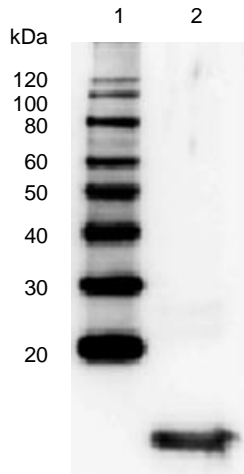
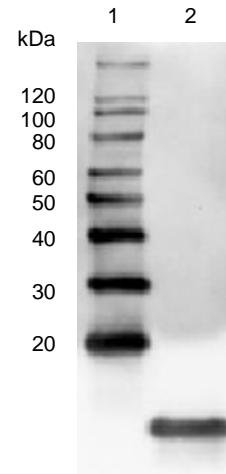


Figure 2: Western Blot with Polyclonal Anti-ESAT-6



Lane 1: MagicMark™ XP Protein Standard  
Lane 2: NR-49424, 1.0 µg

Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard  
Lane 2: NR-49424, 1.0 µg

Date: 17 NOV 2017

Signature:   
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