

# **Certificate of Analysis for NR-44223**

## Fraction 1 Capsular Antigen (F1) from Yersinia pestis, Recombinant from Escherichia coli

### Catalog No. NR-44223

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

### **Product Description:**

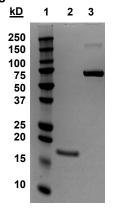
NR-44223 is a recombinant form of the *Yersinia pestis* (*Y. pestis*) fraction 1 capsular antigen (F1). The precursor recombinant protein lacking the native signal sequence but including an N-terminal hexa-histidine tag and a thrombin cleavage site was expressed in *Escherichia coli* and purified by nickel affinity chromatography. Treatment with immobilized bovine thrombin resulted in removal of the N-terminal hexa-histidine tag and the thrombin-cleaved protein was further purified by anion exchange chromatography and another round of nickel affinity chromatography to remove the cleaved tag. The final protein contains amino acid residues 22 to 170 of F1 from *Y. pestis* and four non-F1 residues at the amino terminus.

Lot: 70046227 Manufacturing Date: 18NOV2021

TEST	SPECIFICATIONS	RESULTS
Appearance	Clear and colorless	Clear and colorless
SDS-PAGE Analysis	Protein band of interest represents > 90% of total staining intensity	Dominant band of ~ 18 kDa accounts for >97% of total staining intensity (Figure 1)
Identification by Mass Spectrometry	Measured mass within 5% of theoretical value (15975 daltons)	Measured mass within 0.04% of theoretical value (15968.48 daltons)
Identification by Western Blot Analysis		
Monoclonal anti- Yersinia pestis F1	Reactive	Reactive (Figure 2) <sup>1</sup>
Monoclonal anti-histidine tag	Non-reactive	Non-reactive (Figure 3) <sup>2</sup>
Concentration by Bicinchoninic Acid Assay		
Bovine Serum Albumin (standard)	Report results	0.663 mg per mL
Final Product		
Quantity per vial	Report results	238.7 μg
Volume per vial	Report results	360 µL
Endotoxin Content		
Limulus Amoebocyte Lysate Assay	< 100 EU per mg	< 7.54 EU per mg
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

<sup>&</sup>lt;sup>1</sup>Using a 1:1000 dilution of mouse monoclonal anti-Yersinia pestis F1 (Santa Cruz Biotechnology SC-52303) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems HAF007) as secondary antibody

Figure 1: SDS-PAGE Analysis



Lane 1: Precision Plus Protein™ Standard

Lane 2: NR-44223 (1 μg)

Lane 3: BSA (1 µg)

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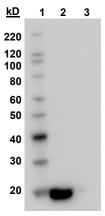
Fax: 703-365-2898

<sup>&</sup>lt;sup>2</sup>Using a 1:1000 dilution of mouse monoclonal anti-histidine tag (Takara 631212) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems HAF007) as secondary antibody



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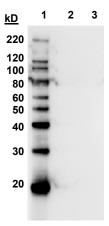
Figure 2: Western Blot with Monoclonal Anti-Yersinia pestis F1



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-44223 (1 μg) Lane 3: BSA (1 μg)

Figure 3: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-44223 (1 μg) Lane 3: BSA (1 μg)

/Sonia Bjorum Brower/ Sonia Bjorum Brower

10 JUN 2022

Lead Technical Writer or designee, ATCC Federal Solutions

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