

Certificate of Analysis for NR-43783

N2 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/turkey/Wisconsin/1/1966 (H9N2), Recombinant from Baculovirus

Catalog No. NR-19237

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

Product Description:

A recombinant form of the N2 neuraminidase (NA) protein from influenza A virus, A/turkey/Wisconsin/1/1966 (H9N2) containing an N-terminal histidine tag was produced in Sf9 insect cells using a baculovirus expression vector system and purified by nickel affinity chromatography. The predicted ectodomain coding region of the NA gene was fused to a synthetic gene segment encoding an N-terminal octa-histidine tag followed by a 43 amino acid tetramerization domain from vasodilator-stimulated phosphoprotein (VASP) and a thrombin cleavage site, as described for the 1918 pandemic virus.

Lot: 70050535 Manufacturing Date: 18MAR2022

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 ~60 kDa accounts for ~98.2% of total staining intensity (Figure 1)
Identification by Western Blot Analysis		
Monoclonal anti-histidine tag ¹	Reactive	Reactive (Figure 2A)
Polyclonal anti-N2 NA ²	Reactive	Reactive (Figure 2B)
Concentration by Bradford Assay	Report results	479 μg per mL
Final Product		
Quantity per vial	Report results	158 μg
Volume per vial	Report results	330 μL
Functional Activity Neuraminidase activity in fluorescent enzymatic assay³	Report results	1.33 × 10 ¹¹ relative fluorescence units per hour per mg protein
Endotoxin Content (Limulus Amoebocyte Lysate Assay)	Report results	< 1.44 EU per mg
Filtration	0.2 μm sterile-filtered	0.2 µm sterile-filtered

¹Using a 1:2000 dilution of mouse monoclonal anti-histidine tag (R&D Systems, Cat. No. MAB050) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems, Cat. No. HAF007) as secondary antibody

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²Using a 1:2000 dilution of goat polyclonal anti-A/Singapore/1/57 (BEI Resources, NR-3137) as primary antibody and a 1:1000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Cat. No. HAF109) as secondary antibody

³Using serial dilutions of NR-19237 and 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (4-MUNANA), as described in Wetherall, N. T., et al. "Evaluation of Neuraminidase Enzyme Assays Using Different Substrates to Measure Susceptibility of Influenza Virus Clinical Isolates to Neuraminidase Inhibitors: Report of the Neuraminidase Inhibitor Susceptibility Network." J. Clin. Microbiol. 41 (2003): 742-750. PubMed: 12574276.



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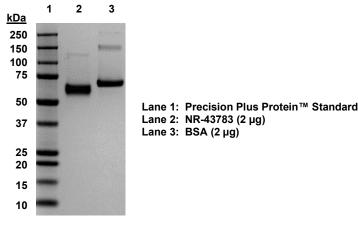
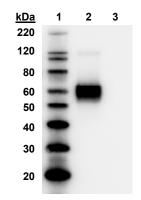


Figure 2: Western Blot Analysis

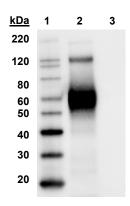
A: Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-43783 (0.5 μg) Lane 3: BSA (0.5 μg)

B: Polyclonal Anti-N2 Neuraminidase



Lane 1: MagicMark™ XP Protein Standard

Lane 2: NR-43783 (0.5 μg) Lane 3: BSA (0.5 μg)

/Sonia Bjorum Brower/ Sonia Bjorum Brower

02 DEC 2022

Technical Manager or designee, ATCC Federal Solutions

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