Certificate of Analysis for NR-19234

N1 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/California/04/2009(H1N1)pdm09, 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 N1 neuraminidase (NA) protein from influenza A virus, A/California/04/2009(H1N1)pdm09 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: 70051971 Manufacturing Date: 19AUG2022

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 50-60 kDa accounts for ~ 90% of total staining intensity (Figure 1)
Identification by Western Blot Analysis Monoclonal anti-histidine tag[1] Polyclonal anti-N1 NA[2]	 Reactive Reactive	 Reactive (Figure 2A) Reactive (Figure 2B)
Concentration by Bradford Assay	Report results	106 µg per mL
Final Product Quantity per vial Volume per vial	 Report results Report results	 53 µg 500 µL
Functional Activity Neuraminidase activity in fluorescent enzymatic assay[3]	Report results	3.49 × 10^8 relative fluorescence units/hour/mg protein
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

[1]Using a 1:1000 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

[2]Using a 1:1000 dilution of goat polyclonal anti-A/New Jersey/8/1976 (H1N1) (BEI Resources, NR-3136) as primary antibody and a 1:1000 dilution of HRP-conjugated donkey anti-goat IgG (R&D Systems, Cat. No. HAF109) as secondary antibody

[3]Using 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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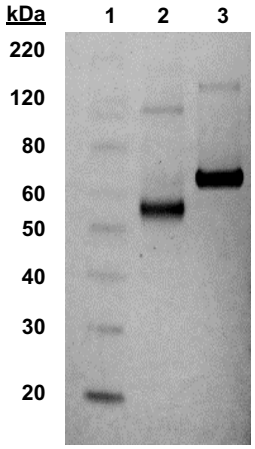
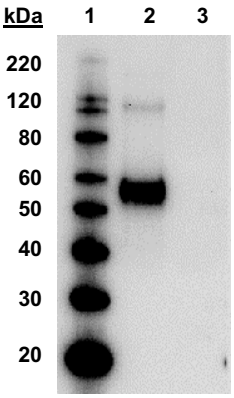
Figure 1: SDS-PAGE Analysis



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-19234 (1 µg)
Lane 3: BSA (1 µg)

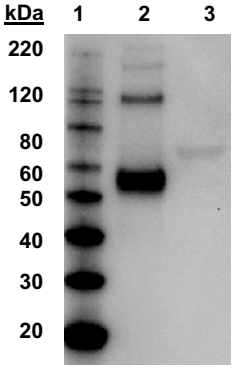
Figure 2: Western Blot Analysis

A: Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-19234 (0.5 µg)
Lane 3: BSA (0.5 µg)

B: Polyclonal Anti-N1 Neuraminidase



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-19234 (0.5 µg)
Lane 3: BSA (0.5 µg)

/Sonia Bjorum Brower/
Sonia Bjorum Brower 11 SEP 2023
Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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