Certificate of Analysis for NR-43785

N1 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/Brisbane/59/2007 (H1N1), Recombinant from Baculovirus

Catalog No. NR-43785

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/Brisbane/59/2007 (H1N1) 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 eight-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. NR-43785 lot 70043130 was vialed in 16 mM Na_2HPO_4, 400 mM NaCl (pH 7.5) with 20% glycerol.

Lot: 70043130 **Manufacturing Date: 16SEP2021**

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 kDa accounts for > 90% of total staining intensity (Figure 1)
Identification by Western Blot Analysis Monoclonal anti-histidine tag Polyclonal anti-influenza A virus, A/Brisbane/59/2007 (H1N1)	 Reactive Reactive	 Reactive (Figure 2)[1] Reactive (Figure 3)[2]
Concentration by Bradford Assay Bovine Serum Albumin (BSA; standard)	 Report results	 171 µg per mL
Final Product Quantity per vial Volume per vial	 Report results Report results	 89.78 µg 525 µL
Functional Activity Neuraminidase activity in fluorescent enzymatic assay	Report results	4.5×10^7 relative fluorescence units per hour per mg protein
Endotoxin Content Limulus Amoebocyte Lysate Assay	 Report results	 < 29.23 EU per mg
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

[1]Using a 1:5000 dilution of mouse monoclonal anti-histidine tag (R&D Systems MAB050) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-mouse IgG (R&D Systems HAF007) as secondary antibody

[2]Using a 1:500 dilution of ferret polyclonal anti-A/Brisbane/59/2007 (BEI Resources NR-19260) as primary antibody and a 1:1000 dilution of HRP-conjugated goat anti-ferret IgG (Abcam ab112770) as secondary antibody

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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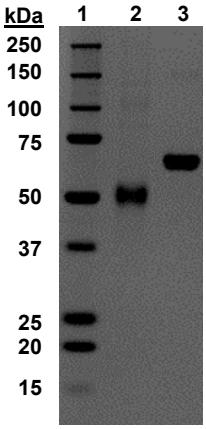
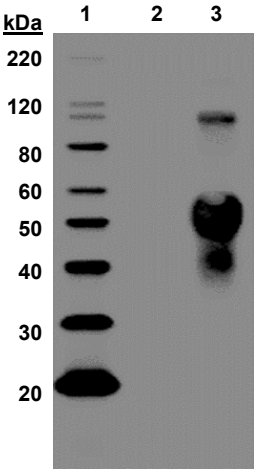
Figure 1: SDS-PAGE Analysis



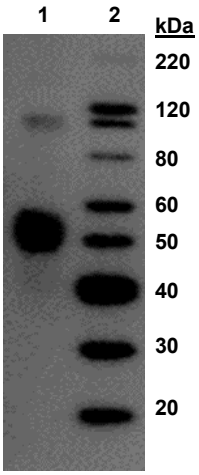
Lane 1: Bio-Rad Precision Plus Protein™ Standard
Lane 2: NR-43785 (0.5 µg)
Lane 3: BSA (0.5 µg)

Figure 2: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: Invitrogen™ MagicMark™ XP Protein Standard
Lane 2: BSA (0.5 µg)
Lane 3: NR-43785 (0.5 µg)

Figure 3: Western Blot with Polyclonal Anti-Influenza A Virus



Lane 1: NR-43785 (0.5 µg)
Lane 2: Invitrogen™ MagicMark™ XP Protein Standard

/Sonia Bjorum Brower/
Sonia Bjorum Brower 03 OCT 2022
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

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898