Certificate of Analysis for NR-28607

H1 Hemagglutinin (HA) Protein with C-Terminal Histidine Tag from Influenza Virus, A/Brisbane/59/2007 (H1N1), Recombinant from Baculovirus

Catalog No. NR-28607

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

Product Description:

A recombinant form of the H1 hemagglutinin (HA) protein from influenza A virus, A/Brisbane/59/2007 (H1N1) was produced in High Five™ insect cells using a baculovirus expression vector system and was purified by nickel affinity chromatography. NR-28607 lacks the signal sequence and contains 501 residues (ectodomain) of the influenza A virus, A/Brisbane/59/2007 (H1N1); the recombinant protein includes a thrombin cleavage site, T4 foldon trimerization domain and C-terminal octa-histidine tag, as described for the 1918 pandemic virus. Semi-purified protein from the previous lot (70001607) which was stored at -20°C was purified by nickel affinity chromatography, washed with Triton™ X-114 (1%), dialyzed into PBS, adjusted to contain 50% (v:v) glycerol, sterile-filtered and aliquoted into vials to produce this lot.

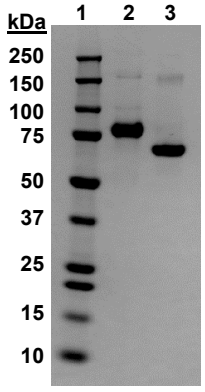
Lot: 70048698 **Manufacturing Date: 15NOV2021**

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 ~ 80 kDa accounts for 95.3% of total staining intensity (Figure 1)
Identification by Western Blot Analysis		
Monoclonal anti-histidine tag	Reactive	Reactive (Figure 2)[1]
Polyclonal anti-HA	Reactive	Reactive (Figure 3)[2]
Concentration by Bradford Assay		
Bovine Serum Albumin (standard)	Report results	484 µg per mL
Final Product		
Quantity per vial	Report results	106 µg
Volume per vial	Report results	220 µL
Functional Activity		
Hemagglutination activity		
0.5% chicken red blood cells	Report results	Negative
0.5% turkey red blood cells	Report results	Positive (1:400)
Endotoxin Content		
Limulus Amoebocyte Lysate Assay	Report results	< 20.66 EU per mg
Filtration	0.2 µm sterile-filtered	0.2 µm sterile-filtered

[1]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

[2]Using a 1:1000 dilution of rabbit polyclonal anti-HA (H1N1)(A/New Caledonia/20/99) (Immune Technology IT-003-001) as primary antibody and a 1:1000 dilution of HRP-conjugated mouse anti-rabbit IgG (Santa Cruz Biotechnology SC-2357) as secondary antibody

Figure 1: SDS-PAGE Analysis



Lane 1: Precision Plus Protein™ Standard
Lane 2: NR-28607 (1 µg)
Lane 3: BSA (1 µg)

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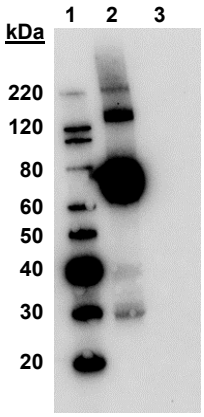
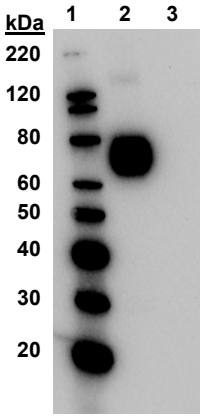
Figure 2: Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-28607 (1 µg)
Lane 3: BSA (1 µg)

Figure 3: Western Blot with Polyclonal Anti-HA



Lane 1: MagicMark™ XP Protein Standard
Lane 2: NR-28607 (1 µg)
Lane 3: BSA (1 µg)

/Heather Couch/
Heather Couch 25 MAY 2022
Program Manager or designee, ATCC Federal Solutions

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