b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-42974

H3 Hemagglutinin (HA) Protein with C-Terminal Histidine Tag from Influenza Virus, A/Perth/16/2009 (H3N2), Recombinant from Baculovirus

Catalog No. NR-42974

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

Product Description: A recombinant form of the H3 hemagglutinin (HA) protein from influenza virus A/Perth/16/2009 (H3N2) was produced by baculovirus infection of *Trichoplusia ni* insect larvae purified by standard chromatographic methods.

Lot: 61489532 **Manufacturing Date: 28MAR2013**

TEST (Required)	SPECIFICATIONS	RESULTS
SDS-PAGE (Coomassie Blue Densitometry)	HA0 band accounts for > 90% of total density	HA0 band accounts for 99% of total density (Figure 1)
Concentration by Bicinchoninic Acid Protein Assay	Report results	1.04 mg per mL
Final Product Quantity per vial	0.1 mg (± 10%) per vial	0.1 mg (± 10%) per vial
Western Blot Anti-H3 monoclonal antibody[1] Anti-histidine monoclonal antibody[2]	Report results Report results	Reactive with HA0 (Figure 2) Reactive with HA0 (Figure 3)
Demonstration of Protein Glycosylation	Size reduction of protein observed on SDS-PAGE when treated with de-glycosylating enzymes N-glycosidase F	Size reduction of protein observed on SDS-PAGE when treated with de-glycosylating enzyme N-glycosidase F (Figure 4)
Filtration	0.22 µm filtered	0.22 µm filtered
Bioburden	Report results	< 1 colony-forming unit per mL
Endotoxin Content	Report results	< 0.05 EU per mL

[1]Abcam ab66187
[2]Novagen® 70796-3 (IgG_1)

Date: 14 MAY 2013 **Signature:** Dorothy C. Young

Title: Technical Manager, BEI Authentication or designee

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by the contractor 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.

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Support Provided by NIAID

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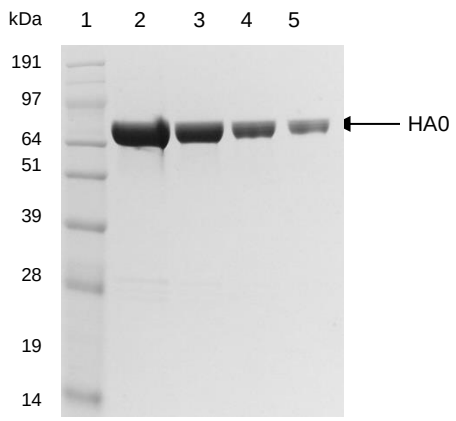
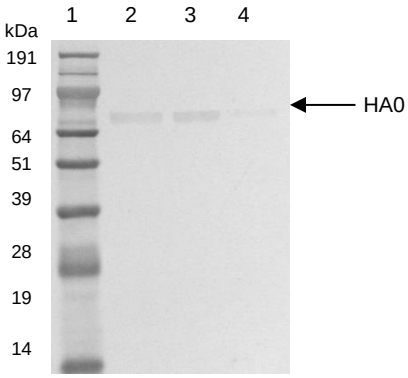
Figure 1 – SDS-PAGE



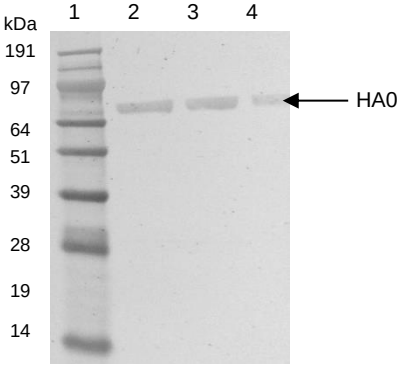
Lane 1: SeeBlue® Plus2 MW marker (Invitrogen™ LC5925)
Lane 2: 10.0 µg NR-42974
Lane 3: 5.0 µg NR-42974
Lane 4: 2.5 µg NR-42974
Lane 5: 1.25 µg NR-42974

Figure 2 - Western Blot with Monoclonal Anti-H3



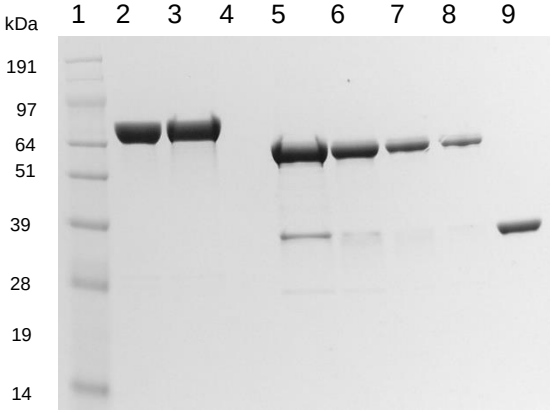
Lane 1: SeeBlue® Plus2 MW marker (Invitrogen™ LC5925)
Lane 2: 1.0 µg NR-42974
Lane 3: 0.5 µg NR-42974
Lane 4: 0.25 µg NR-42974

Figure 3 - Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: SeeBlue® Plus2 MW marker (Invitrogen™ LC5925)
Lane 2: 1.0 µg NR-42974
Lane 3: 0.5 µg NR-42974
Lane 4: 0.25 µg NR-42974

Figure 4 – Glycan Analysis



Lane 1: SeeBlue® Plus2 MW marker (Invitrogen™ LC5925)
Lane 2: 5 µg NR-42974
Lane 3: 7 µg NR-42974 Negative PNGaseF
Lane 4: Blank
Lane 5: 6.7 µg NR-42974, PNGaseF Digest
Lane 6: 3.3 µg NR-42974, PNGaseF Digest
Lane 7: 1.7 µg NR-42974, PNGaseF Digest
Lane 8: 0.83 µg NR-42974, PNGaseF Digest
Lane 9: PNGaseF Enzyme

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