

## **Certificate of Analysis for NR-42487**

# H1 Hemagglutinin (HA) Protein with C-Terminal Histidine Tag from Influenza Virus, A/Christchurch/16/2011 (H1N1)pdm09, Recombinant from Baculovirus

### Catalog No. NR-42487

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

**Product Description:** A recombinant form of the H1 hemagglutinin (HA) protein from influenza virus A/Christchurch/16/2011 (H1N1)pdm09 containing a C-terminal histidine tag was produced by baculovirus infection of *Trichoplusia ni* insect larvae and purified by standard chromatographic methods.

Lot: 61376138 Manufacturing Date: 01FEB2013

| TEST   | SPECIFICATIONS   | RESULTS   |
|--|--|---|
| SDS-PAGE (Coomassie Blue Densitometry)                       | HA bands account for > 90% of total density  | HA bands account for > 93% of total density (Figure 1)                              |
| Concentration by Bicinchoninic Acid<br>Protein Assay         | Report results   | 0.55 mg per mL  |
| Final Product  Quantity per vial                             | 0.1 mg (± 10%) per vial  | 0.1 mg (± 10%) per vial   |
| Hemagglutination Titer with 0.5%<br>Chicken Red Blood Cells  | Report results   | 32,768 <sup>1</sup>   |
| Western Blot Anti-histidine monoclonal antibody <sup>2</sup> | Report results   | Reactive with HA0 and HA2 (Figure 2)  |
| Demonstration of Protein<br>Glycosylation                    | Size reduction of protein observed on<br>SDS-PAGE when treated with de-<br>glycosylating enzyme N-glycosidase<br>(PNGaseF) | Size reduction of protein observed on SDS-PAGE when treated with PNGaseF (Figure 3) |
| 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  |

<sup>&</sup>lt;sup>1</sup>Highest dilution of protein that showed hemagglutination activity

**Date:** 25 APR 2013

Signature:

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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BEI Resources www.beiresources.org E-mail: contact@beiresources.org

Tel: 800-359-7370 Fax: 703-365-2898

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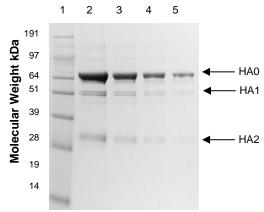
<sup>&</sup>lt;sup>2</sup>Novagen<sup>®</sup> 70796-4 (IgG<sub>1</sub>)



#### SUPPORTING INFECTIOUS DISEASE RESEARCH

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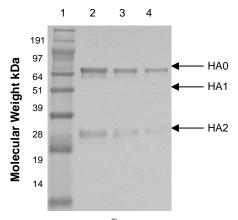
Figure 1 – SDS-PAGE



Lane 1: SeeBlue® Plus2 MW marker

(Invitrogen™ LC5925) Lane 2: 5.0 µg NR-42487 Lane 3: 2.5 µg NR-42487 Lane 4: 1.25 µg NR-42487 Lane 5: 0.63 µg NR-42487

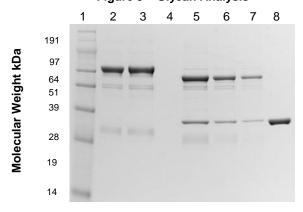
Figure 2 - Western Blot with Monoclonal Anti-Histidine Tag



Lane 1: SeeBlue® Plus2 MW marker

(Invitrogen™ LC5925) Lane 2: 1.0 µg NR-42487 Lane 3: 0.5 µg NR-42487 Lane 4: 0.25 µg NR-42487

Figure 3 - Glycan Analysis



Lane 1: SeeBlue® Plus2 MW marker (Invitrogen™ LC5925)

Lane 2: 5 µg NR-42487

Lane 3: 5 µg NR-42487 with digest mixture without PNGaseF

Lane 4: Blank

Lane 5: 5 µg NR-42487, PNGaseF Digest Lane 6: 2.5 µg NR-42487, PNGaseF Digest Lane 7: 1.25 µg NR-42487, PNGaseF Digest

Lane 8: 1.5 µg PNGaseF Enzyme