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SUPPORTING INFECTIOUS DISEASE RESEARCH

N1 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/Puerto Rico/8/1934 (H1N1), Recombinant from Baculovirus

Catalog No. NR-42002

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Contributor and Manufacturer:

BEI Resources

Product Description:

A recombinant form of the N1 neuraminidase (NA) protein from influenza A virus, A/Puerto Rico/8/1934 (H1N1) containing an N-terminal histidine tag was produced in insect cells using a baculovirus expression vector system. Lot No. 61759226, which is no longer available, was produced in High FiveTM insect cells and enriched from culture supernatants by nickel affinity chromatography under nondenaturing conditions. Lot No. 63195703 was produced in *Spodoptera frugiperda* Sf9 cells and purified by nickel affinity chromatography under non-denaturing conditions.

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)¹ and a thrombin cleavage site, as described for the 1918 pandemic virus.² The predicted protein sequence is shown in Table 1. The full-length NA precursor protein is 454 residues (GenPept: ABD77678).

NR-42002 was expressed from the same recombinant baculovirus vector as NR-19235, which was purified from cell lysates under denaturing conditions and has not been tested for enzymatic activity.

Material Provided:

Each vial contains approximately 1 to 5 μ g of recombinant NA protein in 25 mM phosphate buffer (pH 8.0) with 250 mM NaCl, 250 mM imidazole, and 50% glycerol. The protein content in μ g and the concentration, expressed as μ g per mL, are shown on the Certificate of Analysis.

Packaging/Storage:

Purified recombinant NA protein was packaged aseptically in screw-capped plastic cryovials. This product is provided on blue ice and should be stored at -20°C immediately upon arrival.

Functional Activity:

NR-42002 was demonstrated to be functionally active based on its ability to cleave the fluorogenic substrate 2'-(4methylumbelliferyl)- α -D-N-acetylneuraminic acid (4-MUNANA).³

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: N1 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/Puerto Rico/8/1934 (H1N1), Recombinant from Baculovirus, NR-42002."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see <u>www.cdc.gov/biosafety/publications/bmbl5/index.htm</u>.

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References:

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- Xu, X., et al. "Structural Characterization of the 1918 Influenza Virus H1N1 Neuraminidase." <u>J. Virol.</u> 82 (2008): 10493-10501. PubMed: 18715929.
- 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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Table 1 – Predicted Protein Sequence

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1	ADPHHHHHHH	H <u>SSSDYSDLQ</u>	<u>RVKQELLEEV</u>	<u>KKELQKVKEE</u>	<u>IIEAFVQELR</u>
51	<u>KRGS</u> LVPRGS	PSRSEFVILT	GNSSLCPIRG	WAIYSKDNSI	RIGSKGDVFV
101	IREPFISCSH	LECRTFFLTQ	GALLNDKHSS	GTVKDRSPYR	ALMSCPVGEA
151	PSPYNSRFES	VAWSASACHD	GMGWLTIGIS	GPDNGAVAVL	KYNGIITETI
201	KSWRKKILRT	QESECACVNG	SCFTIMTDGP	SDGLASYKIF	KIEKGKVTKS
251	IELNAPNSHY	EECSCYPDTG	KVMCVCRDNW	HGSNRPWVSF	DQNLDYQIGY
301	ICSGVFGDNP	RPEDGTGSCG	PVYVDGANGV	KGFSYRYGNG	VWIGRTKSHS
351	SRHGFEMIWD	PNGWTETDSK	FSVRQDVVAM	TDWSGYSGSF	VQHPELTGLD
401	CMRPCFWVEL	IRGRPKEKTI	WTSASSISFC	GVNSDTVDWS	WPDGAELPFS
451	IDK				

Plasmid-derived amino acids – Residues 1 to 3 and 61 to 66 His Tag – Residues 4 to 11 Tetramerization domain – <u>Residues 12 to 54</u> Thrombin cleavage sequence – Residues 55 to 60 NA protein – **Residues 67 to 453***

*This represents amino acid residues 68 to 454 of the A/Puerto Rico/8/1934 (H1N1) NA protein.