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SUPPORTING INFECTIOUS DISEASE RESEARCH

N1 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/California/04/2009 (H1N1)pdm09, Recombinant from Baculovirus

Catalog No. NR-19234

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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/California/04/2009 (H1N1)pdm09 containing an N-terminal histidine tag was produced in Sf9 insect cells using a baculovirus expression vector system and was 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)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 453 residues (GenPept: ACP44158).

Material Provided:

Each vial contains approximately 50 μ g of purified recombinant NA protein in PBS (pH 7.4). The protein content in μ g and the concentration, expressed as μ g/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 frozen and should be stored at -20°C or colder immediately upon arrival. For long-term storage, freezing at -80°C or colder is recommended. Multiple freeze-thaw cycles should be avoided.

Functional Activity:

NR-19234 was demonstrated to be functionally active based on its ability to cleave the fluorogenic substrate 2'-(4-methylumbelliferyl)- 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/California/04/2009 (H1N1)pdm09, Recombinant from Baculovirus, NR-19234."

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:

- Kühnel, K., et al. "The VASP Tetramerization Domain is a Right-Handed Coiled Coil Based on a 15-Residue Repeat." <u>Proc. Natl. Acad. Sci. USA</u> 101 (2004): 17027-17032. PubMed: 15569942.
- Xu, X., et al. "Structural Characterization of the 1918 Influenza Virus H1N1 Neuraminidase." J. Virol. 82 (2008): 10493-10501. PubMed: 18715929.
- 3. Wetherall, N. T., et al. "Evaluation of Neuraminidase Enzyme Assays Using Different Substrates to Measure

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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

1	ADPHHHHHHH	H <u>SSSDYSDLQ</u>	<u>RVKQELLEEV</u>	<u>KKELQKVKEE</u>	<u>IIEAFVQELR</u>
51	<u>KRGS</u> LVPRGS	PSRSEF VKLA	GNSSLCPVSG	WAIYSKDNSV	RI GSKGDVFV
101	I REPFI SCSP	LECRTFFLTQ	GALLNDKHSN	GTI KDRSPYR	TLMSCPIGEV
151	PSPYNSRFES	VAWSASACHD	GINWLTIGIS	GPDNGAVAVL	KYNGIITDTI
201	KSWRNNI LRT	QESECACVNG	SCFTVMTDGP	SNGQASYKI F	RIEKGKIVKS
251	VEMNAPNYHY	EECSCYPDSS	EITCVCRDNW	HGSNRPWVSF	NQNLEYQI GY
301	I CSGI FGDNP	RPNDKTGSCG	PVSSNGANGV	KGFSFKYGNG	VWIGRTKSIS
351	SRNGFEMI WD	PNGWTGTDNN	FSI KQDI VGI	NEWSGYSGSF	VQHPELTGLD
401	CIRPCFWVEL	I RGRPKENTI	WTSGSSI SFC	GVNSDTVGWS	WPDGAELPFT
451	I DK				

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 83 to 469 of the A/California/04/2009 (H1N1)pdm NA protein.