

N8 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/equine/Pennsylvania/1/2007 (H3N8), Recombinant from Baculovirus

Catalog No. NR-13523

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Contributor and Manufacturer:

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Product Description:

The N8 neuraminidase (NA) protein from influenza A virus, A/equine/Pennsylvania/1/2007 (H3N8) containing an N-terminal histidine tag was produced in High-Five™ insect cells using a baculovirus expression vector system and was purified by metal 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 N8 NA precursor protein is 463 residues (GenPept: BA168051).

Material Provided:

Each vial contains approximately 50 to 100 µg of purified recombinant NA protein in 25 mM phosphate buffer (pH 7.0) with 500 mM NaCl (Lot 59948172) or in PBS (pH 7.4) with 50% glycerol (Lot 6223222). The protein content in µg and the concentration, expressed as µg 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 frozen and should be stored at -20°C immediately upon arrival. For long-term storage, freezing at -80°C or colder is recommended. Although enzymatic activity was not affected by a single cycle of freezing at -80°C and thawing, multiple freeze-thaw cycles should be avoided.

Functional Activity:

NR-13523 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:

N8 Neuraminidase (NA) Protein with N-Terminal Histidine Tag from Influenza Virus, A/equine/Pennsylvania/1/2007 (H3N8), Recombinant from Baculovirus, NR-13523."

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. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Kühnel, K., et al. "The VASP Tetramerization Domain is a Right-Handed Coiled Coil Based on a 15-residue Repeat." Proc. Natl. Acad. Sci. USA 101 (2004): 17027-17032. PubMed: 15569942.

2. 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 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	HSSSDYSDLQ	RVKQELLEEV	KKELQKVKEE	IIEAFVQELR
51	KRGS ^U LVPRGS	PSRSEFY ^U YMN	NTEPLCEAQG	FAPFSK ^U NGI	RIGSRGHV ^U FV
101	IREFV ^U SCSP	SECR ^U TFFLTQ	GSL ^U LNDKHSN	GTVKDR ^U SPYR	TLMSV ^U KIGQS
151	PNVY ^U QARFES	VAWSA ^U TACHD	GKKW ^U MTVGVT	GPDNQ ^U AI ^U AVV	NYGG ^U VPVDII
201	NSWAG ^U DILRT	QESS ^U CTCIK ^U G	DCYW ^U VMTDGP	ANRQA ^U KYRIF	KAKDGR ^U VIGQ
251	TDIS ^U FNGGHI	EECS ^U CYPNEG	KVEC ^U ICRD ^U NW	TGTNR ^U PILVI	SSDL ^U SYTVGY
301	LCAG ^U IPTDTP	RGED ^U GQFTGS	CTNPL ^U G ^U NKGY	GVKGF ^U GFRQG	TDVW ^U AGRTIS
351	RTSR ^U SGFEII	KIRNG ^U WTQNS	KDQ ^U IRRQVII	DDPN ^U WSGYSG	SFTLP ^U VELTK
401	KECL ^U VPCFWV	EMIR ^U GKPEET	TIWT ^U SSSSIV	MCGV ^U DHKIAS	WSWH ^U DGAILP
451	FDID ^U KM				

Plasmid-derived amino acids – Residues 1 to 3 and 61 to 66

His Tag – Residues 4 to 11

Tetramerization domain – Residues 12 to 54

Thrombin cleavage sequence – Residues 55 to 60

NA protein – Residues 67 to 456*

*This represents amino acid residues 74-463 of the A/equine/Pennsylvania/1/2007 (H3N8) NA protein, which is identical to A/equine/Yokohama/aq19/2009 (GenPept: BAI68051) except for a serine to glycine change at position 322 of the neuraminidase precursor protein (position 315 of the recombinant protein).