**DESCRIPTION**

**Source**
Spodoptera frugiperda, Sf 21 (baculovirus)-derived

**N-terminal Sequence Analysis**
Ser (of tetramerization domain)

**Predicted Molecular Mass**
53 kDa

**SPECIFICATIONS**

**SDS-PAGE**
57-65 kDa, reducing conditions

**Activity**
Measured by its ability to cleave a fluorogenic substrate, 2-(4-Methylumbelliferyl)-o-D-N-acetylmuramidic acid. The specific activity is >10,000 pmol/min/μg, as measured under the described conditions.

**Endotoxin Level**
<1.0 EU per 1 μg of the protein by the LAL method.

**Purity**
>85%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

**Formulation**
Supplied as a 0.2 μm filtered solution in Tris, NaCl, CaCl₂, DTT and Glycerol. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**
- Assay Buffer: 25 mM MES, 500 mM NaCl, 5 mM CaCl₂, pH 6.5
- Recombinant Influenza A Virus H3N2 Neuraminidase (rvH3N2) (Catalog # 6875-NM)
- Substrate: 4-Methylumbelliferyl-o-D-N-acetylmuramidic acid (Sigma, Catalog # M8639), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (SpectraMax Gemini EM by Molecular Devices) or equivalent

**Assay**
1. Dilute rvH3N2 Neuraminidase to 0.2 ng/μL in Assay Buffer.
2. Dilute Substrate to 400 μM in Assay Buffer.
3. Load 50 μL of the 0.2 ng/μL rvH3N2 Neuraminidase into a black well plate and start the reaction by adding 50 μL of 400 μM Substrate. Include a Substrate Blank containing Assay Buffer in place of rvH3N2 Neuraminidase.
4. Read at excitation and emission wavelengths of 365 nm and 445 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:
   
   \[
   \text{Specific Activity (pmol/min/μg)} = \frac{\text{Adjusted } V_{\text{max}}^* \times (\text{RFU/min}) \times \text{Conversion Factor}^{**}}{\text{amount of enzyme (μg)}}
   \]

   *Adjusted for Substrate Blank
   **Derived using calibration standard 4-Methylumbelliferone (Sigma, Catalog # M1381)

**Final Assay Conditions**
Per Well:
- rvH3N2 Neuraminidase: 0.010 μg
- Substrate: 200 μM

**PREPARATION AND STORAGE**

**Shipping**
The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

**BACKGROUND**

Neuraminidase (NA) and hemagglutinin (HA) are the two predominant membrane glycoproteins found on the surface of an influenza virus particle. They are essential for the infectious cycle of the virus. HA recognizes and binds to the sialic acid on the host cell membrane to initiate a viral infection. NA cleaves the sialic acid at the end of the cycle, allowing the progeny virus to leave the host thus initiating the next round of infection (1). In the early stage of an infection, NA may also assist in viral penetration of the mucus layer in the airway of a host. Nine subtypes of NA (N1 to N9) have been identified, all of which are believed to be tetrameric and share a basic structure consisting of a globular head, a thin stalk region, and a small hydrophobic region that anchors the protein in the virus membrane (2). Glycosylation is also found to be important for the stability and activity of these enzymes (3). Due to their critical role in the infectious cycle of a virus, influenza viral neuraminidases are frequently used as targets for drug design. Both the anti-influenza drugs Tamiflu® and Relenza® are neuraminidase inhibitors. According to a recent structure determination (4), neuraminidases from influenza type A viruses form two genetically distinct groups, with the N1 and N2 neuraminidases representing each of the two groups. This recombinant protein is based on the sequences of the virus isolated from 1968 Hong Kong flu pandemic (5). An artificial tetramerization domain from the vasodilator-stimulated phosphoprotein (6) was inserted at the N-terminus to assist in oligomerization of the recombinant protein.

**References**