**DESCRIPTION**

**Source**  
Spodoptera frugiperda, Sf 21 (baculovirus)-derived
Ser37-Lys469, with an N-terminal 6-His tag  
Accession # AAF77036

**N-terminal Sequence Analysis**  
His

**Predicted Molecular Mass**  
48 kDa

**SPECIFICATIONS**

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

**Activity**  
Measured by its ability to cleave a fluorogenic substrate, 2’-(4-Methylumbelliferyl)-o-D-N-acetylneuraminic acid.  
The specific activity is >2,500 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**  
>80%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation**  
Supplied as a 0.2 µm filtered solution in Tris-HCl and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**

- Assay Buffer: 50 mM Tris, 5 mM CaCl₂, 200 mM NaCl, pH 7.5
- Recombinant Influenza A Virus H1N1 Neuraminidase (rH1N1 Neuraminidase) (Catalog # 4858-NM)
- Substrate: 2’-(4-Methylumbelliferyl)-o-D-N-acetylneuraminic acid (Sigma, Catalog # M6639), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

**Assay**

1. Dilute rH1N1 Neuraminidase to 0.05 ng/µL in Assay Buffer.
2. Dilute Substrate to 400 µM in Assay Buffer.
3. Load 50 µL of 0.05 ng/µL rH1N1 Neuraminidase into the plate, and start the reaction by adding 50 µL of 400 µM Substrate. As a Substrate Blank load 50 µL of Assay Buffer and 50 µL of Substrate.
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:

\[
\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\max} \times (RFU/min) \times \text{Conversion Factor} (\text{pmol/RFU})}{\text{amount of enzyme (µg)}}
\]

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

**Final Assay Conditions**

- Per Well:
  - rH1N1 Neuraminidase: 0.0025 µg
  - Substrate: 200 µM

**PREPARATION AND STORAGE**

**Shipping**

The product is shipped with polar packs. 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, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

**BACKGROUND**

Neuraminidase (NA) and hemagglutinin (HA) are major membrane glycoproteins found on the surface of influenza virus. HA is a lectin that binds sialic acid on host cell membrane. NA is a sialic acid hydrolase that specifically clips off terminally located sialic acid on host cell surface. The two proteins are essential for the infectious cycle of the influenza virus. During initial infection, an influenza virus will hold onto an epithelial cell through HA-sialic acid interaction. At the end of an infectious cycle, the NA will cleave the sialic acid on the host cell membrane, releasing the formed viral particle from the HA-sialic acid bondage (1). The neuraminidase activity is also thought to help the virus penetrate mucus. Nine subtypes of NA have been identified, all of which are tetrameric and share a common structure consisting of a globular head, a thin stalk region, and a small hydrophobic region that anchors the protein in the virus membrane (2). The purified rH1N1NA consists of amino acid residues 37 to 469 as deduced from the 1918 Spanish flu virus NA (A/Bervig_Mission/1/18) (3). It has a distinct N-glycan profile and is resistant to trypsin digestion (4).

**References:**