

## EnzyChrom™ Neuraminidase Assay Kit (ENEU-100)

### Quantitative Colorimetric/Fluorimetric Determination of Neuraminidase Activity

#### DESCRIPTION

**NEURAMINIDASE** (also known as Sialidase) is an enzyme that hydrolyzes terminal sialic acid residues on poly-saccharide chains. It is predominantly expressed in microorganisms such as bacteria and viruses. Neuraminidase is believed to play several roles in infection by influenza viruses and is thus an important target for influenza drug development. Simple, direct and automation-ready procedures for measuring neuraminidase activity find wide applications in research and drug discovery. BioAssay Systems' neuraminidase assay measures the N-acetylneuraminic acid (NANA) released by neuraminidase in one step. The change in color intensity of the reaction product at 570nm or fluorescence intensity at  $\lambda_{em/ex} = 585/530nm$  is directly proportional to neuraminidase activity in the sample.

#### KEY FEATURES

**Sensitive and accurate.** Linear detection range at 37°C in 96-well plate: 0.1 to 10 U/L for colorimetric assays and 0.01 to 2 U/L for fluorimetric assays.

**Simple and convenient.** Homogeneous assay requiring only two absorbance measurements. Assay can be completed in 60 min.

**High-throughput.** Can be readily automated as a high-throughput 96-well plate assay to screen thousands of samples per day.

#### APPLICATIONS:

**Direct Assays:** neuraminidase activity in biological samples.

**Drug Discovery:** evaluation of neuraminidase inhibitors.

#### KIT CONTENTS (100 tests in 96-well plates)

**Assay Buffer:** 6 mL    **Substrate:** 6 mL    **Cofactors:** 120  $\mu$ L  
**Dye Reagent:** 60  $\mu$ L    **Enzyme:** 120  $\mu$ L    **Standard:** 500  $\mu$ L

**Storage conditions.** The kit is shipped on ice. Store all reagents at -20°C. Shelf life of 6 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

##### COLORIMETRIC PROCEDURE

**Note:** SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to desired reaction temperature (i.e. 37°C). Prepare a 400  $\mu$ M Standard Premix by mixing 20  $\mu$ L of the 10 mM Standard and 480  $\mu$ L dH<sub>2</sub>O. Dilute Standard in distilled water as follows.

No	Premix + H <sub>2</sub> O	Sialic Acid ( $\mu$ M)
1	50 $\mu$ L + 0 $\mu$ L	400
2	30 $\mu$ L + 20 $\mu$ L	240
3	15 $\mu$ L + 35 $\mu$ L	120
4	0 $\mu$ L + 50 $\mu$ L	0

Transfer 20  $\mu$ L standards into separate wells of a clear flat-bottom 96-well plate.

2. Transfer 20  $\mu$ L of each sample into two separate wells of the same plate. One well will be used for the sample activity and one for the sample blank.
3. Immediately prior to starting the reaction, prepare enough Working Reagent (WR) for all sample and standard wells by mixing per reaction tube: 30  $\mu$ L Assay Buffer, 55  $\mu$ L Substrate, 1  $\mu$ L Cofactors, 1  $\mu$ L Enzyme and 0.5  $\mu$ L Dye Reagent. For the sample blank wells, substitute 55  $\mu$ L Assay Buffer for the 55  $\mu$ L Substrate. Add 80  $\mu$ L of the appropriate WR to each well.

4. Incubate the reaction plate protected from light at 37°C (or desired temperature) for 20 min. Measure the OD at 570 nm ( $OD_{20min}$ ). Incubate reaction plate for a further 30 min, again protected from light and at 37°C (or desired temperature). Measure the OD ( $OD_{50min}$ ).

#### FLUORIMETRIC PROCEDURE

1. Dilute the Standards prepared in *Colorimetric Procedure* 1:5 in H<sub>2</sub>O. Transfer 20  $\mu$ L standards into separate wells of a *black* 96-well plate.
2. Transfer 20  $\mu$ L of each sample into two separate wells of the same plate. One well will be used for the sample activity and one for the sample blank.
3. Add 80  $\mu$ L of appropriate Working Reagent (see *Colorimetric Procedure*) to each well. Tap plate to mix.
4. Incubate the reaction plate protected from light at 37°C (or desired temperature) for 20 min. Measure the F ( $\lambda_{ex/em} = 530/570$  nm) ( $F_{20min}$ ). Incubate reaction plate for a further 30 min, again protected from light and at 37°C (or desired temperature). Measure the F ( $F_{50min}$ ).

#### CALCULATION

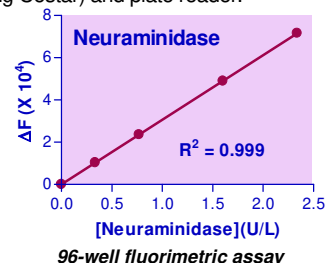
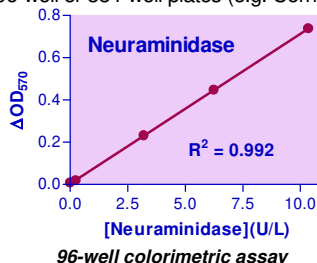
Plot the OD or F measured at 50 min for each standard against the standard concentrations. Determine the slope using linear regression fitting. Subtract the optical density or fluorescence values for the 20 min time point from the values of the 50 min time point for the sample, sample blank and H<sub>2</sub>O (water, #4) reactions. The neuraminidase activity of a Sample is calculated as

$$\text{Neuraminidase Activity} = \frac{\Delta R_{\text{SAMPLE}} - \Delta R_{\text{BLANK}} - \Delta R_{\text{H}_2\text{O}}}{\text{Slope}} \times \frac{1}{t} \quad (\text{U/L})$$

where  $\Delta R_{\text{SAMPLE}}$ ,  $\Delta R_{\text{BLANK}}$  and  $\Delta R_{\text{H}_2\text{O}}$  are the changes in optical density or fluorescence values of the sample, sample blank and H<sub>2</sub>O (water, #4) respectively. **Slope** is the slope of the standard curve in  $\mu\text{M}^{-1}$  and **t** is the time of reaction between readings (30 min). 1 Unit (U) of neuraminidase will catalyze the release of 1  $\mu$ mole of NANA per minute under assay conditions. **Note:** if the Sample activity is higher than the 10 U/L for the colorimetric assay or 2 U/L for the fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well or 384-well plates (e.g. Corning Costar) and plate reader.



#### PUBLICATIONS

1. Lida, M. et al (2019). A sialo-oligosaccharide-rich mucin-like molecule specifically detected in the submandibular glands of aged mice. *Archives of oral biology*, 97, 52-58.
2. Subathra, M et al (2014). Evaluation of antibody response in mice against avian influenza A (H5N1) strain neuraminidase expressed in yeast *Pichia pastoris*. *J Biosci*. 39(3):443-51.

