

EnzyChrom™ Neuraminidase Assay Kit (ENEU-100)

Quantitative Colorimetric/Fluorimetric Determination of Neuraminidase Activity

DESCRIPTION

NEURAMINDASE (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. Cleavage of sialic acid residues by neuraminidase is believed to play several roles in infection by influenza viruses. It is thought to assist in the penetration of mucosal linings, the invasion of target cells, the elution of progeny viruses from infected cells, and the prevention of self-aggregation. Thus, neuraminidase is an important target for influenza drug development and simple, direct and automation-ready procedures for measuring neuraminidase activity find wide applications in research and drug discovery. BioAssay Systems' neuraminidase assay measures the sialic acid released by neuraminidase in one step. The change in color intensity of the reaction product at 570nm or fluorescence intensity at $\lambda_{em}/\lambda_{ex} = 585/530\text{nm}$ 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 μL

Dye Reagent: 60 μL **Enzyme:** 120 μL **Standard:** 500 μL

Storage conditions. The kit is shipped on ice. Store all reagents at - 20°C. Shelf life of three 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 µM Standard Premix by mixing 20 µL of the 10 mM Standard and 480 µL dH₂O. Dilute Standard in distilled water as follows.

No	Premix + H ₂ O	Sialic Acid (µM)
1	50 µL + 0 µL	400
2	30 µL + 20 µL	240
3	15 µL + 35 µL	120
4	0 µL + 50 µL	0

Transfer 20 µL standards into separate wells of a clear flat-bottom 96-well plate.

2. Transfer 20 µL 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 µL Assay Buffer, 55 µL Substrate, 1 µL Cofactors, 1 µL Enzyme and 0.5 µL Dye Reagent. For the sample blank wells, substitute 55 µL Assay Buffer for the 55 µL Substrate. Add 80 µL 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 H₂O. Transfer 20 µL standards into separate wells of a *black* 96-well plate.

2. Transfer 20 µL 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 µL 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 (lex/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

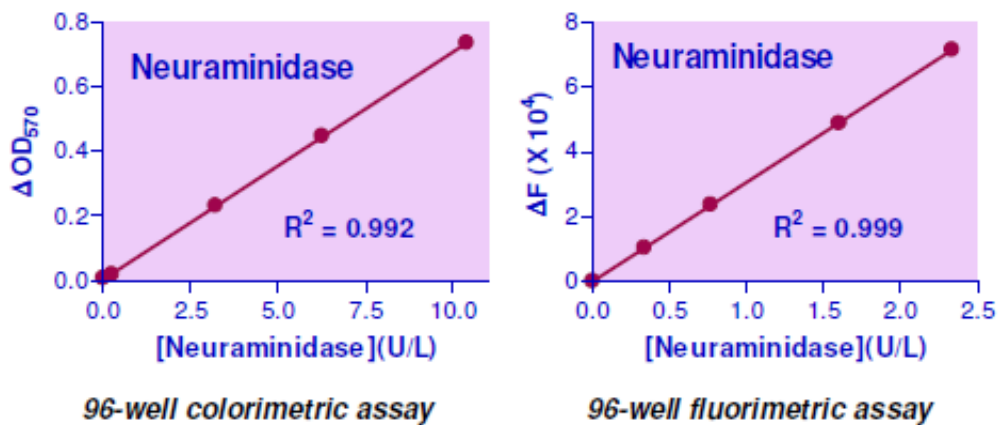
Plot the OD or F measured at 50 min for each standard against the standard concentrations. Determine the slope using linear regression fitting. Subtract the optical density or fluorescence values for the 20 min time point from the values of the 50 min time point for the sample, sample blank and H₂O (water, #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 **_RSAMPLE**, **_RBLANK** and **_RH2O** are the changes in optical density or fluorescence values of the sample, sample blank and H₂O (water, #4) respectively. **Slope** is the slope of the standard curve in μM^{-1} and t is the time of reaction between readings (30 min). *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.



LITERATURE

1. Sugahara, K. et al. (1980). Enzymatic Assay of Serum Sialic Acid. Clinica Chimica Acta 108:493-8.
2. Simpson, H. et al. (1993). Serum sialic acid enzymatic assay based on microtitre plates: application for measuring capillary serum sialic acid concentrations. Br J Biomed Sci. 50:164-7.