Tribioscience

Neuraminidase Activity Fluorometric Assay (Catalog: TBS2109, Store at -20°C)

Description

Neuraminidase (NA, EC 3.2.1.18) is a type of glycoside hydrolase enzyme present in many organisms. It cleaves sialic acid residues from glycoproteins and glycolipids. This action is essential for many biological processes. It plays a critical role in viral replication and cellular processes, particularly in influenza virus infection. Therefore, viruses are often classified by neuraminidase subtypes, such as N1, N2, etc. Neuraminidase is related to neurodegenerative disease, such as Alzheimer's disease, and Parkinson's disease.

The Neuraminidase Activity Fluorometric Assay provides a simple, accurate, and sensitive method for monitoring neuraminidase activity in biological samples. In this assay, neuraminidase cleaves a non-fluorescent substrate to release a fluorescent product, which can be measured at an excitation wavelength of 365 nm and an emission wavelength of 445 nm.

Synonyms: Sialidase; NA; Acyl-neuraminyl hydrolase, Receptor-destroying enzyme; N-Acylneuraminate Glycohydrolases.

Applications

- Neuraminidase activity in biological samples.
- Infuenza virus antigen titration.

Key Features

Simplicity: The assay directly detects enzymatic activity without complex enzyme coupling steps.
Sensitivity: Even low levels of neuraminidase activity can be detected due to the high fluorescence intensity.

Kit Contents for 100 Assays

Component	Part Size
Substrate (50X)	120 µL
Standard (1 mM)	50 µL
Positive control (12X)	10 µL
Stop Reagent	12 mL
Assay Buffer	15 mL

Storage and Shipment Conditions

The kit is shipped on ice and should be stored at -20°C.

Procedures

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Substrate and Stop Reagent to samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

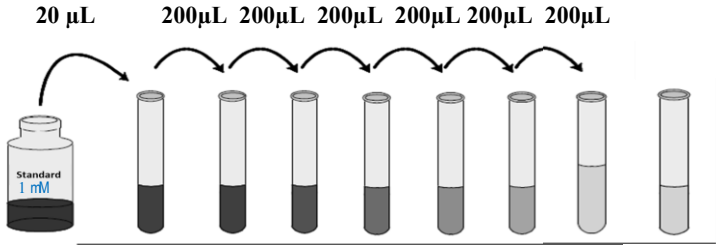
Sample Preparation:

Use a suitable approach to prepare samples.
Reagent Preparation: Equilibrate all components to room temperature. Briefly vortex or pipette up and down all components to ensure the components are fully mixed.

Reaction Preparation:

1. Prepare the substrate working solution by diluting 50-fold of the substrate stock, e.g. add 120 µL of substrate stock into 5.88 mL assay buffer for 96-well plate.
2. Prepare 1x positive control: add 10 µL Positive Control to 110 µL assay buffer.
3. Label tubes as #1 through #8 as below diagram.
4. Add 380 µL of 1x Assay Buffer to Std1, and 200 µL of 1xAssay Buffer to Std2 to 8.
5. Pipet 20 µL of 1 mM standard stock into Std#1. Then, make 2x series dilution in Std2 through 7 with addition of 200 µL. Std8 is 1x Assay Buffer alone as a standard 0. The standard concentration in tube 1 through 7 will be 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 µM (2500, 1250, 625, 312.5, 156.25,78.125,39.0625, 0 pmol/well), Tube#8 is Standard 0 as blank. Showed in Fig.1 as below.

Fig. 1: Standard preparation.



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	380	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	20	200	200	200	200	200	200	0
Final Conc (µM)	50	25	12.5	6.25	3.125	1.56	0.78	0

6. Transfer 50 µL of each sample, blank, positive control or standards into the indicated wells of a black 96-well plate in duplicate style.
7. Add 50 µL of the substrate working solution to the sample, positive control, and blank wells. Add 50 µL of the assay buffer to the standards. *(Note: Do not add substrate in the standard*). Tap plate briefly to mix.
8. Incubate at 37°C for 1-2 hours gently shaking, protecting from light (Longer incubation has a stronger activity).
9. Add 100 µL of Stop Reagent to all wells. Tap plate briefly to mix.
10. Read at Ex/Em = 365 nm/445 nm in different time point.

Calculation

Subtract blank RFU (Standard 0, #8) from the standard RFU values and plot the ΔRFU against standard concentrations. Determine the slope, and use the following equation to calculate NAase activity:

NA Activity (Unit/µM/min) = DF * (RFU SAMPLE – RFU BLANK)/ (t * Slope)

where RFU SAMPLE is the measurement for each sample and RFU BLANK is the fluorescent value of the sample blank. Slope is the slope of the linear regression fit of the standard points and t is the reaction time (60 min or other time). DF is the dilution factor.

Unit definition: 1 Unit (U) will catalyze the conversion of 1 µM of non-fluorescent substrate to fluorescent product per min at 37°C.

Typical Data

Standard curve: This standard curve in Fig 2 is provided as a reference demonstration. A standard curve should be generated for each set of samples assayed.

Fig.2: Standard Curve

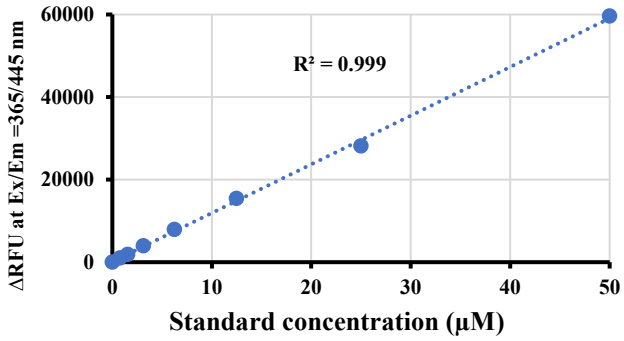
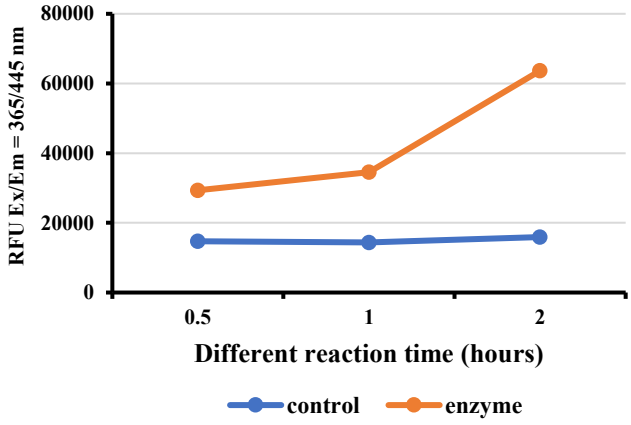


Fig.3 displays the NA enzyme activity analyzed at different time points of incubation at 37°C.

Fig.3: Neuraminidase activity assay (4µg/ml/well)



Related Products:

LDH Cytotoxicity colorimetric Assay (TBS2002)
Tryptase Activity Assay (TBS2101)
B-N-acetylglucosaminidase Activity Colorimetric Assay (TBS2105)
Caspase-3 Fluorometric Assay kit (TBS3230)
Cytochrome C Oxidase Activity Assay (TBS2115)
Fast Glucose Determination Colorimetric/Fluorometric Assay (TBS2087)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay (TBS2088)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
Protein Assay Kits (TBS2005)
Cell Nuclear Extract kit (TBS6025)

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