Amplite™ Fluorimetric Neuraminidase Assay Kit *Blue Fluorescence*

Catalog number: 12602
Unit size: 200 Tests

Component	Storage	Amount
Component A: FluLite™ Blue	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: Neuraminidase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (0.1 U)

OVERVIEW

Neuraminidases, also called sialidases, are glycoside hydrolase enzymes that catalyze the hydrolysis of terminal sialic acid residues and neuraminic acid. The most commonly known neuraminidase is the viral neuraminidase. The cleavage of linkage between sialic acid and adjacent sugar residue permits the transport of the virus through mucin and destroys the haemagglutinin receptor on the host cell, thus allowing elution of progeny virus particles from infected cells. Neuraminidase promotes influenza virus release from infected cells and facilitates virus spread within the respiratory tract. Thus, it is an important target for influenza drug development. The detection of neuraminidase and screening its inhibitors is one of the essential tasks for investigating biological processes and prevention of influenza infection. There are a few assay kits available for detecting neuraminidase, but all the commercial available kits are tedious to use. Our Amplite™ Fluorimetric Neuraminidase Assay Kit provides a sensitiveand robust fluorimetric assay to detect neuraminidase that exists either in cells or biological samples. The non-fluorescent neuraminidase substrate becomes strongly fluorescent upon neuraminidase cleavage. The kit can detect as little as 0.3 mU/mL neuraminidase in a 100 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The signal can be easily read by a fluorescence microplate reader.

AT A GLANCE

Protocol summary

1. Prepare Neuraminidase working solution (50 µL)
2. Add Neuraminidase standards or test samples (50 µL)
3. Incubate at 37°C or room temperature for 1 - 2 hours
4. Monitor fluorescence increase at Ex/Em = 320/460 nm (Cutoff = 420 nm)

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	320 nm
Emission:	460 nm
Cutoff:	420 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Neuraminidase standard solution (2U/mL):
Add 50 µL of ddH_2O into the vial of Neuraminidase Standard (Component C) to make approximately 2 U/mL Neuraminidase standard solution.

2. FluLite™ Blue stock solution (200X):
Add 50 µL of ddH_2O into the vial of FluLite™ Blue (Component A) to make 200X stock solution.

PREPARATION OF STANDARD SOLUTION

Neuraminidase standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/12602

Add 10 µL of 2 U/mL Neuraminidase standard stock solution to 990 µL of Assay Buffer (Component B) to generate 20 mU/mL Neuraminidase standard (NA7). Take 20 mU/mL Neuraminidase standard solution and perform 1:2 serial dilutions to get serially diluted Neuraminidase standards (NA6 - NA1) with Assay Buffer (Component B).

Note Diluted Neuraminidase standard solution is unstable. Use within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 25 µL of 200X FluLite™ Blue stock solution into 5 mL of Assay Buffer (Component B) and mix well to prepare Neuraminidase working solution.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of neuraminidase standards and test samples in a solid black 96-well microplate. NA= Neuraminidase Standards (NA1 - NA7, 0.312 to 20 mU/mL), BL=Blank Control, TS=Test Samples.

BL	BL	TS	TS
NA1	NA1	...	...
NA2	NA2	...	...
NA3	NA3		
NA4	NA4		
NA5	NA5		
NA6	NA6		
NA7	NA7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
NA1 - NA7	50 µL	Serial Dilution (0.312 to 20 mU/mL)
BL	50 µL	Blank Control
TS	50 µL	test sample

1. Prepare Neuraminidase standards (NA), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of Neuraminidase working solution to each well of Neuraminidase standard, blank control, and test samples to make the total Neuraminidase assay volume of 100 µL/well. For a 384-well plate, add 25 µL of Neuraminidase working solution into each well instead, for a total volume of 50

µL/well.

3. Incubate the reaction at 37°C or room temperature for 1 to 2 hours, protected from light.

   Note 37°C incubation gives better results.

4. Monitor the fluorescence increase at Ex/Em = 320/460 nm (Cutoff = 420 nm) with a fluorescence microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Neuraminidase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

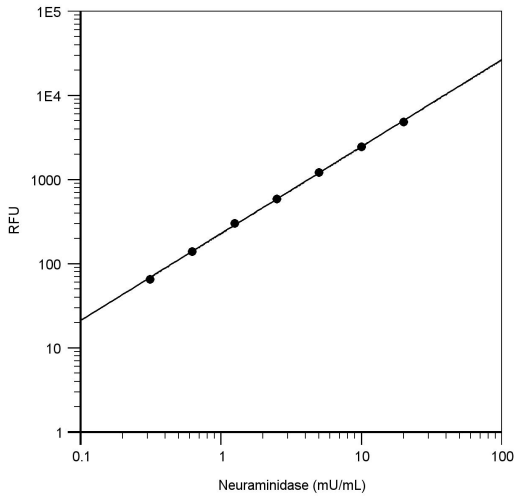

Figure 1. Neuraminidase dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Neuraminidase Assay Kit using a Gemini fluorescence microplate reader (Molecular Devices).

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