Amplite™ Fluorimetric Neuraminidase Assay Kit

Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 12602 (200 assays)	Keep at -20 °C Avoid moisture and light	Fluorescence microplate readers

Introduction

Neuraminidases, also called sialidases, are glycoside hydrolase enzymes that catalyze the hydrolysis of terminal sialic acid residues and neuraminic acids. 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 Ex/Em = ~320/~450 nm.

Kit Key Features

Broad Application:	Can be used for quantifying neuraminidase in a variety of biological fluids and cells.
Sensitive:	Detect as low as 0.3 mU/mL neuraminidase.
Continuous:	Easily adapted to automation without a separation step.

Kit Components

Components	Amount
Component A: FluLite™ Blue	1 vial
Component B: Assay Buffer	1 bottle (20 mL)
Component C: Neuraminidase Standard	1 vial (0.1 U)

Assay Protocol for One 96-well Plate

Brief Summary

Prepare FluLite™ Blue reaction mixture (50 µL) → Add neuraminidase standards or test samples (50 µL) → Incubate at 37 °C or room temperature for 1-2 hours→ Monitor fluorescence increase at Ex/Em = ~320/~450 nm

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

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1. Prepare neuraminidase standard stock solution:

Add 50 µL of ddH_2O into the vial of Neuraminidase Standard (Component C) to make 2 U/mL neuraminidase standard stock solution.
Note: The concentration of this stock solution is approximately 2U/mL. The unused Neuraminidase Standard solution should be divided into single use aliquots and stored at -20 °C.

2. Prepare 200X FluLite™ Blue stock solution:

Add 50 µL of ddH_2O into the vial of FluLite™ Blue (Component A) to make 200X stock solution.
Note: The unused FluLite™ Blue solution should be divided into single use aliquots and stored at -20 °C and kept from light.

3. Prepare neuraminidase assay mixture:

Add 25 µL of 200X FluLite™ Blue stock solution (from Step 2) into 5 mL of Assay Buffer (Component B), and mix well.

4. Prepare serial dilutions of neuraminidase standard (0 to 20 mU/mL):

4.1 Add 10 µL of 2U/mL neuraminidase standard stock solution (from Step 1) to 990 µL of assay buffer (Component B) to generate 20 mU/mL neuraminidase standard.
Note: Diluted neuraminidase standard solution is unstable. Use within 4 hours.

4.2 Take 500 µL of 20 mU/mL neuraminidase standard solution (from Step 4.1) to perform 1:2 serial dilutions to get 10, 5, 2.5, 1.25, 0.625, 0.312 and 0 mU/mL serial dilutions of neuraminidase standard.

4.3 Add neuraminidase standards and neuraminidase -containing test samples into a solid black 96-well microplate as shown in Tables 1 and 2.

Table 1 Layout of neuraminidase standards and test samples in a solid black 96-well microplate

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

Note: NA= NA Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

NA Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

**Note: Add the serial dilutions of neuraminidase standard from 0.312 mU to 20 mU into wells from NA1 to NA7 in duplicate.*

5. Run neuraminidase assay:

5.1 Add 50 µL of neuraminidase assay mixture (from Step 3) to each well of the neuraminidase standard, blank control, and test samples (see Step 4.3) to make the total neuraminidase assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of neuraminidase reaction mixture into each well.

5.2 Incubate the reaction at 37 °C or room temperature for 1 to 2 hours, protected from light.
Note: 37°C incubation gives better results.

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

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the neuraminidase reactions. A neuraminidase standard curve is shown in Figure 1.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

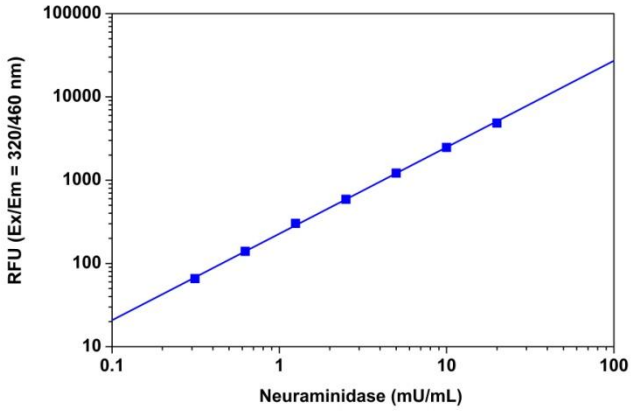

Figure 1. Neuraminidase dose response was measured in a 96-well black plate with Amplite™ Fluorimetric Neuraminidase Assay Kit using a Germini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/mL of neuraminidase can be detected with 1 hour incubation time in 37°C, 5% CO_2 incubator.

References

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