ab138888

Neuraminidase Assay Kit (Fluorometric-Blue)

Instructions for Use

For the detection of neuraminidase in cells or biological fluids.

This product is for research use only and is not intended for diagnostic use.

Version 4 Last Updated 27 February 2019

Table of Contents

1. 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 commercially available kits are tedious to use.

ab138888 provides a sensitive and robust fluorometric assay to detect neuraminidase that exists either in cells or biological samples. The non-fluorescent neuraminidase substrate becomes strongly fluorescent upon neuraminidase cleavage. ab138888 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

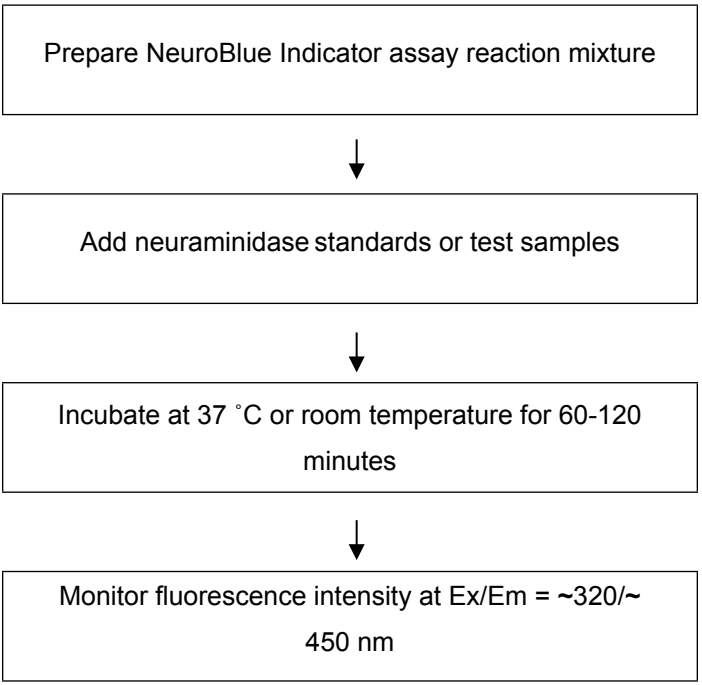
separation step. The signal can be easily read by a fluorescence microplate reader at Ex/Em = ~320/~450 nm.

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.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

Components	Amount
Component A: NeuroBlue Indicator	1 vial
Component B: Assay Buffer	1 x 20 mL
Component C: Neuraminidase Standard	1 x 0.1 U

4. Storage and Handling

Keep at -20°C. Avoid exposure to moisture and light.

5. Additional Materials Required

- 96 or 384-well solid black microplates
- Fluorescence microplate reader
- ddH_2O

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. 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 2 U/mL. The unused Neuraminidase Standard solution should be divided into single use aliquots and stored at -20 °C.

B. Prepare 200X NeuroBlue Indicator stock solution: Add 50 µL of ddH_2O into the vial of NeuroBlue Indicator (Component A) to make 200X stock solution.

Note: The unused NeuroBlue indicator solution should be divided into single use aliquots, stored at -20 °C and kept from light.

C. Prepare neuraminidase assay reaction mixture: Add 25 µL of 200X NeuroBlue stock solution (from Step B) into 5 mL of Assay Buffer (Component B), and mix well.

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

1. Add 10 µL of neuraminidase standard stock solution (Step A) to 990 µL of Assay Buffer (Component B) to generate 20 mU/ml neuraminidase standard.
   Note: Diluted neuraminidase standard solution is unstable and should be used within 4 hours.
2. Take 500 µL of 20 mU/mL neuraminidase standard solution to perform 1:2 serial dilutions with Assay Buffer (Component B) to generate 10, 5, 2.5, 1.25, 0.625, 0.312 and 0 mU/mL serial dilutions of neuraminidase standard.
3. Add serially diluted neuraminidase standards and/or neuraminidase containing test samples into a solid black 96-well microplate as described in Tables1 and 2.

E. Sample preparation:

1. Tissue or cells samples: 20-50 mg of tissue or 1 million cells should be rapidly homogenized with 100 µl of ice cold PBS or other buffer (pH 6.5-8). Centrifuge at 13000 rpm for 10 min to remove insoluble materials.
2. Liquid or solution samples: can be directly diluted in the Assay Buffer.

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

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

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

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

Table 2. Reagent composition for each well.

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

F. Run neuraminidase assay:

1. Add 50 µL of neuraminidase assay reaction mixture (Step C) to each well of the neuraminidase standard, blank control, and test samples 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 in each well.

2. Incubate the reaction at 37 ˚C or room temperature for 60 to 120 minutes protected from light.

Note: 37°C incubation gives better results.

3. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 320/450 nm (cutoff = 420 nm).

7. 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.

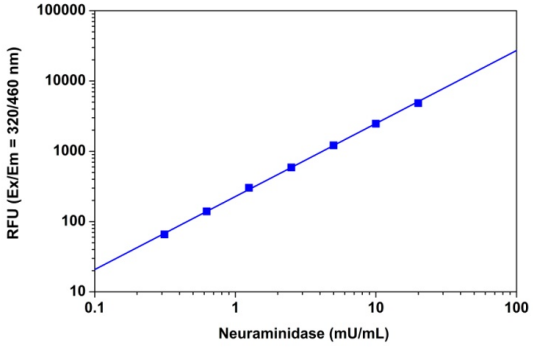


Figure 1. Neuraminidase dose response was measured in a 96-well black plate with Neuraminidase Assay Kit (Fluorometric –Blue) using a Germini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/mL of neuraminidase can be detected with 1 hour incubation time in 37^{o}C, 5% CO_2 incubator.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)

	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes

	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp