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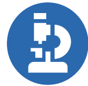 Laborgeräte & Service

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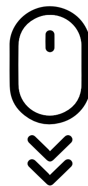

## Lieferung & Zahlungsart

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**SZABO-SCANDIC HandelsgmbH**

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

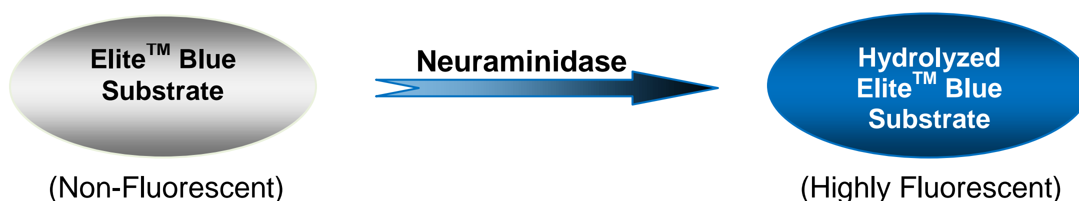
linkedin.com/company/szaboscandic

# Elite™ Neuraminidase Activity Assay Kit (Blue Fluorescence)

CATALOG NUMBER: CA-N602, 200 assays

## Description

Neuraminidases, also called sialidases, catalyze the hydrolysis of terminal sialic acid residues from the newly formed virions and from the host cell receptors. 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 hemagglutinin 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.




(Non-Fluorescent) (Highly Fluorescent)

Our Elite™ Neuraminidase Activity Assay Kit provides a sensitive and 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.

## Features

- Wide Applications: can be used for quantifying influenza neuraminidase and other viral or non-viral neuraminidase activity in a variety of biological fluids and cells.
- High Sensitivity: detect as low as 0.3 mU/ml neuraminidase.
- One-Step Performance: avoid the tedious steps in other commercial assay kits by using our Elite™ fluorescent neuraminidase substrate.

## Kit Components

| | |
|---|---|
| • Component A: Elite™ Blue Substrate | 1 vial |
| • Component B: Assay Buffer | 1 bottle (20 ml) |
| • Component C: Neuraminidase Standard | 1 vial (0.1 U) |

## Storage

Keep in freezer (-20 °C) and avoid exposure to light; **thaw all the kit components at room temperature before starting the experiment.**

## Materials Required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader.

## Assay Protocol

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 $^oC$.

2. **Preparation of 200X Elite$^{TM}$ Blue Substrate stock solution:**

   Add 50 µL of $ddH_2O$ into the vial of Elite$^{TM}$ Blue Substrate (Component A) to make 200X stock solution. Note: The unused Elite$^{TM}$ Blue Substrate solution should be divided into single use aliquots and stored at -20 $^oC$ and kept from light.

3. **Preparation of neuraminidase assay mixture:**

   Add 25 µL of 200X Elite$^{TM}$ Blue Substrate stock solution (from Step 2) into 5 mL of Assay Buffer (Component B), and mix well.

4. **Preparation of 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 Standards | Blank Control | Test Sample |
|---|---|---|
| Serial Dilutions: 50 µl | Assay Buffer: 50 µl | 50 µl |

***Note:*** Add the serially diluted neuraminidase standards 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.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***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.

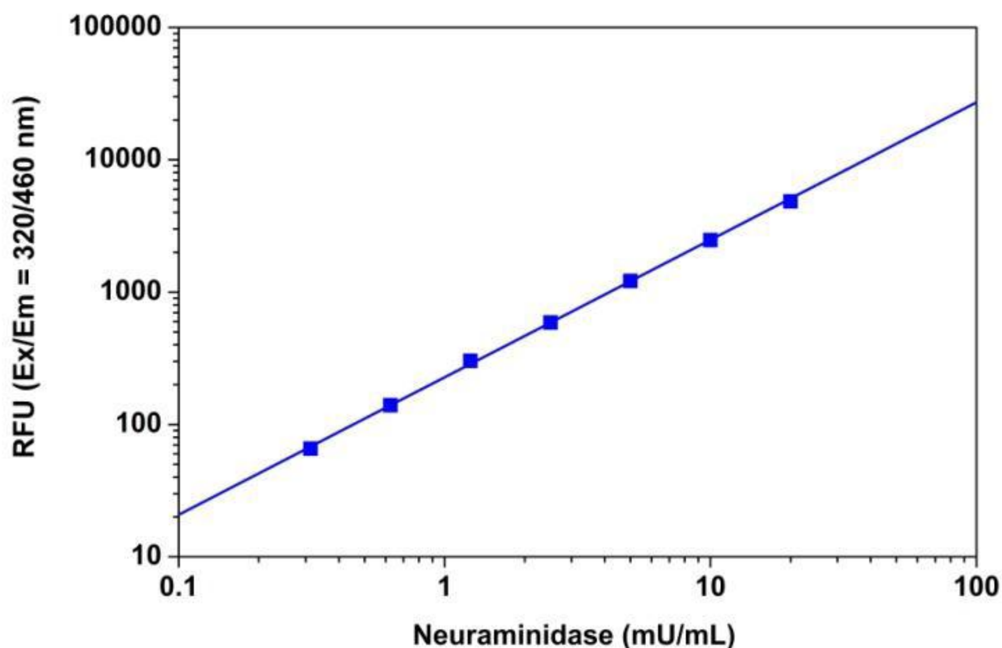


**Figure 1.** Neuraminidase dose response was measured in a 96-well black plate with Elite™ Neuraminidase Assay Kit using a Germini fluorescence microplate reader (Molecular Devices). As low as 0.3 mU/mL of neuraminidase can be detected with 1 hour incubation time in 37 °C, 5% $CO_2$ incubator.

**References:**

1. FA Quiocho, Carbohydrate-binding proteins: tertiary structures and protein-sugar interactions, Annual Review of Biochemistry, 1986, Vol. 55: 287-315, 1986.
2. W. G. Laver, P. M. Colman, R. G. Webster, V. S. Hinshaw and G. M. Air, Influenza virus neuraminidase with hemagglutinin activity, Virology, Vol.137, ( 2), 314-323.
3. JN Varghese, WG Laver, PM Colman, Structure of the influenza virus glycoprotein antigen neuraminidase at 2.9 Å resolution, Nature, 303, 35-40, 1983.
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