Neuraminidase (Sialidase) Assay Kit (Colorimetric/Fluorometric)

LS-K186-100 (100 Tests) • **Store at -20°C**

Introduction

NEURAMINDASE (also known as Sialidase) is an enzyme that hydrolyzes terminal sialic acid residues on poly-saccharide chains. It is predominantly expressed in microorganisms such as bacteria and viruses. Cleavage of sialic acid residues by neuraminidase is believed to play several roles in infection by influenza viruses. It is thought to assist in the penetration of mucosal linings, the invasion of target cells, the elution of progeny viruses from infected cells, and the prevention of self-aggregation. Thus, neuraminidase is an important target for influenza drug development and simple, direct and automation-ready procedures for measuring neuraminidase activity find wide applications in research and drug discovery. This neuraminidase assay measures the sialic acid released by neuraminidase in one step. The change in color intensity of the reaction product at 570nm or fluorescence intensity at $\lambda_{ex/em}$ = 530/585nm is directly proportional to neuraminidase activity in the sample.

Key Features

- Sensitive and accurate. Linear detection range at 37°C in 96-well plate: 0.1 to 10 U/L for colorimetric assays and 0.01 to 2 U/L for fluorometric assays.
- Simple and convenient. Homogeneous assay requiring only two absorbance measurements. Assay can be completed in 60 min.
- High-throughput. Can be readily automated as a high-throughput 96-well plate assay to screen thousands of samples per day.

Applications

- Direct Assays: neuraminidase activity in biological samples.
- Drug Discovery: evaluation of neuraminidase inhibitors.

Components

Component	K186-100
	100 Tests
Assay Buffer	6 mL
Substrate	6 mL
Cofactors	120 µL
Dye Reagent	60 µL
Enzyme	120 µL
Standard	500 µL

Materials Not Supplied

Pipetting devices, centrifuge tubes, Clear flat-bottom 96-well plates, black 96-well or 384-well plates (e.g. Corning Costar) and plate reader.

Storage

The kit is shipped on ice. Store all reagents at -20°C. Shelf life of 6 months after receipt.

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Assay Procedure

Colorimetric Procedure

Note: SH-group containing reagents (e.g. mercaptoethanol, DTT) may interfere with this assay and should be avoided in sample preparation.

1. Equilibrate all components to desired reaction temperature (i.e 37°C). Prepare a 400 µM Standard Premix by mixing 20 µL of the 10 mM Standard and 480 µL dH_2O. Dilute Standard in distilled water as follows.

No	Premix + H_2O	Sialic Acid (µM)
1	50 µL + 0 µL	400
2	30 µL + 20 µL	240
3	15 µL + 35 µL	120
4	0 µL + 50 µL	0

   Transfer 20 µL standards into separate wells of a clear flat-bottom 96-well plate.

2. Transfer 20 µL of each sample into two separate wells of the same

   plate. One well will be used for the sample activity and one for the sample blank.

3. Immediately prior to starting the reaction, prepare enough Working Reagent (WR) for all sample and standard wells by mixing per reaction tube: 30 µL Assay Buffer, 55 µL Substrate, 1 µL Cofactors, 1 µL Enzyme and 0.5 µL Dye Reagent. For the sample blank wells, substitute 55 µL Assay Buffer for the 55 µL Substrate. Add 80 µL of the appropriate WR to each well.

4. Incubate the reaction plate protected from light at 37°C (or desired temperature) for 20 min. Measure the OD at 570 nm (OD_{20min}). Incubate reaction plate for a further 30 min, again protected from light and at 37°C (or desired temperature). Measure the OD (OD_{50min}).

Fluorometric Procedure

1. Dilute the Standards prepared in Colorimetric Procedure 1:5 in H_2O. Transfer 20 µL standards into separate wells of a black 96-well plate.

2. Transfer 20 µL of each sample into two separate wells of the same plate. One well will be used for the sample activity and one for the sample blank.

3. Add 80 µL of appropriate Working Reagent (see Colorimetric Procedure) to each well. Tap plate to mix.

4. Incubate the reaction plate protected from light at 37°C (or desired temperature) for 20 min. Measure the F ($\lambda_{ex/em}$ = 530/570 nm) (F_{20min}). Incubate reaction plate for a further 30 min, again protected from light and at 37°C (or desired temperature). Measure the F (F_{50min}).

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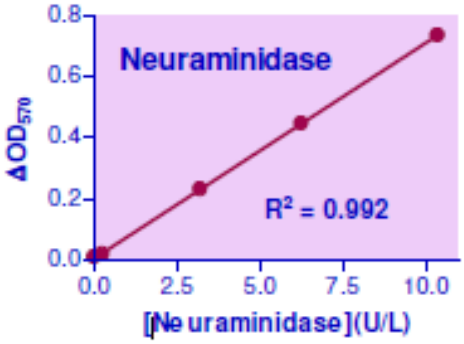
Calculations

Plot the OD or F measured at 50 min for each standard against the standard concentrations. Determine the slope using linear regression fitting. Subtract the optical density or fluorescence values for the 20 min time point from the values of the 50 min time point for the sample, sample blank and H_2O (water, #4) reactions. The neuraminidase activity of a Sample is calculated as

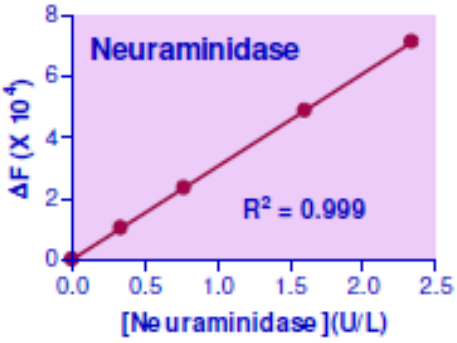
$$\text{Neuraminidase Activity} = \frac{\Delta R_{SAMPLE} - \Delta R_{BLANK} - \Delta R_{H_2O}}{\text{Slope}} \times \frac{1}{t} \quad (\text{U/L})$$

where ΔR_{SAMPLE}, ΔR_{BLANK} and ΔR_{H2O} are the changes in optical density or fluorescence values of the sample, sample blank and H_2O (water, #4) respectively. Slope is the slope of the standard curve in μM^{-1} and *t* is the time of reaction between readings (30 min). Note: if the Sample activity is higher than the 10 U/L for the colorimetric assay or 2 U/L for the fluorometric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

Sample Data



96-well colorimetric assay



96-well fluorimetric assay

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