EnzyChrom™ Neuraminidase Assay Kit (ENU-100)
Quantitative Colorimetric/Fluorimetric Determination of Neuraminidase Activity

DESCRIPTION

Neuraminidase (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. BioAssay Systems' neuraminidase assay measures the sialic acid released by neuraminidase in one step. Sialic acid is an important component of the outer layer of influenza virus that facilitates the attachment of the virus to the cell receptor and is released by the enzyme neuraminidase. The assay measures the sialic acid released by neuraminidase in one step.

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 fluorimetric absorbance measurements. Assay can be completed in 60 min.

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.

KIT CONTENTS (100 tests in 96-well plates)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>6</td>
</tr>
<tr>
<td>Substrate</td>
<td>6</td>
</tr>
<tr>
<td>Cofactors</td>
<td>120</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>60</td>
</tr>
<tr>
<td>Enzyme</td>
<td>120</td>
</tr>
<tr>
<td>Standard</td>
<td>500</td>
</tr>
</tbody>
</table>

FLUORESCENCE PROCEDURE

1. Dilute the Standards prepared in Colorimetric Procedure 1:5 in H₂O. 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 reaction plate protected from light at 37°C (or desired temperature) for 20 min. Measure the F (F<sub>50min</sub>) Incubate reaction plate for a further 30 min, again protected from light and at 37°C (or desired temperature). Measure the F (F<sub>80min</sub>)

COSTABUS SYSTEMS™


FLUORIMETRIC PROCEDURE

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CALCULATION

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 H₂O (water, #4) reactions. The neuraminidase activity of a Sample is calculated as

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

where \( \Delta R_{\text{SAMPLE}} \), \( \Delta R_{\text{BLANK}} \) and \( \Delta R_{\text{H₂O}} \) are the changes in optical density or fluorescence values of the sample, sample blank and H₂O (water, #4) respectively. \( \text{Slope} \) is the slope of the standard curve in pmol 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 fluorimetric assay, dilute sample in water and repeat the assay. Multiply result by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

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

LITERATURE
